BREEDING BIOLOGY OF THE GENUS PSATHYRELLA

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DECLARATION

I hereby declare that all the work presented in this thesis is my own, unless otherwise stated, and that the thesis has been composed by myself.

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ABSTRACT

Psathyrella is a taxonomically difficult genus of the Agaricaceae. Breeding studies were undertaken in an attempt to suggest the reasons for this taxonomic complexity and to provide a basis for a more satisfactory system of classification.

Specimens from the wild were established in culture by the germination of basidiospores. Heat treatment was found to improve spore germination in many isolates.

The incompatibility system was studied in selected isolates. Information on nuclear migration was found essential for the correct determination of the incompatibility system. In some isolates monokaryons which did not accept migrating nuclei were found. In most isolates the incompatibility system was bifactorial, except for one isolate in which the system appeared to be trifactorial. This isolate was investigated in detail since such a system has never been found before.

Light and electron microscope observations were made on oidial structure, in an attempt to resolve the physiological basis for the homing of hyphae towards oidia. The electron microscope studies on P.coprophila showed filamentous appendages on the surface of the oidia. The filaments may be embedded in a mucilagenous capsule and may be similar to 'fimbriae' found on the surface of many bacteria. The function of the filaments and the capsule is not clear. The homing of hyphae towards oidia was observed both within and between isolates. The use of the reaction as a test of relationship between different isolates was developed. In some tests, homing and fusion were followed by a lethal reaction in the hybrid cell. These isolates cannot exchange

genetic information but since plasmogamy does take place they are probably closely related.

The P.'gracilis', the P.'candolleana' and the 'coprophilous' complexes were selected for a further investigation. Within each of these complexes breeding groups were delimited using mycelial crossing tests. Homing responses were used to investigate the degree of relationship. Three breeding groups were found in the P.'gracilis' complex. The homing responses showed that Breeding Groups I and III are more closely related than either is to Breeding Group II, and this was supported by the morphological evidence. Breeding Group II probably corresponds to the morphological species P.microrrhiza. Four breeding groups were delimited in the P.'candolleana' complex. The occurrence of homing and fusion between each of the breeding group combinations suggests that the breeding groups are closely related. The 'coprophilous' isolates can be separated morphologically into the P.'coprobia' and P.'coprophila' complexes. Altogether five coprophilous breeding groups were found. Most of these differed morphologically but more isolates are needed before the breeding groups can be described as separate morphospecies.

In general it is suggested that if no breeding information is available the morphospecies should be wide to allow easy identification using morphological criteria. If breeding information is available it can be used to decide on the diagnostic value of different morphological characters and can also be used to compare the usefulness of existing taxonomic treatments. When breeding groups cannot be distinguished morphologically then they should not be given different formal binomials

even though biologically they are the fundamental units. The shortcomings of the present code of nomenclature for the naming of taxa delimited on breeding evidence are discussed.

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CHAPTER 1 : INTRODUCTION

The biology of toadstools is not a fashionable subject. Most of the current research is directed towards taxonomy or genetics, rarely to a combination of these two subjects. The taxonomists describe and classify the variety of toadstools found in nature. The geneticists, attracted by the elegance of the incompatibility systems are concerned with gene structure and function and models of gene action. On the border line of taxonomy and genetics are several interesting topics which have not been extensively studied. Why are there so many species? What is meant by the term 'species'? Is variation genetically determined or is it mostly environmental? What brings about speciation? These are questions which will never be fully answered, but much valuable evidence can be collected and discussed. Some of the higher fungi are particularly well suited to such a study as their short life cycle, fast growth rate and the relatively undemanding culture conditions provide an opportunity to investigate many of the problems experimentally.

The genus Psathyrella was chosen for the following reasons. Many species are commonly found in Britain, and some members of the genus have already been successfully grown in culture (Quintanilha, Quintanilha and Vasermanis, 1941; Vandendries and Brodie, 1933). The taxonomy of the genus is difficult and new biological information might result in a more satisfactory system of classification.

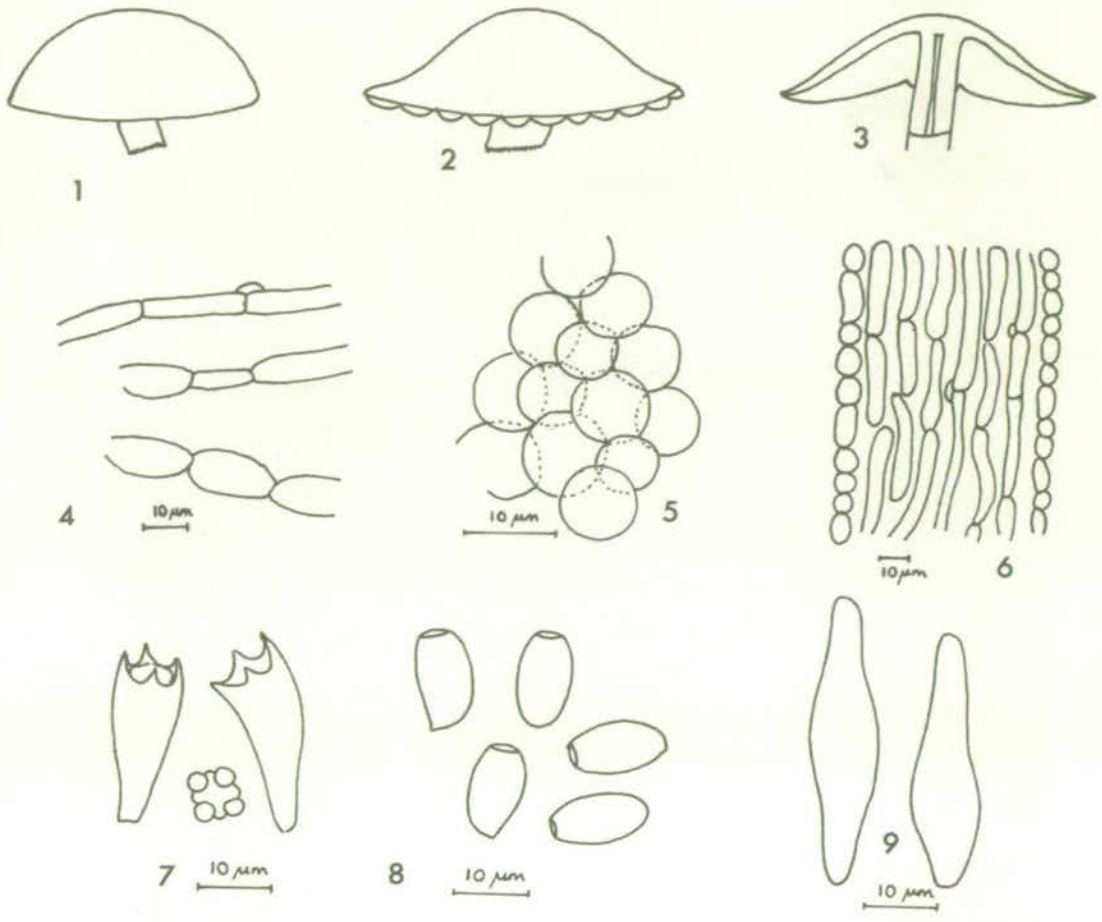
Psathyrella is a genus of small, dark-spored toadstools of the family Coprinaceae. Members of the family differ from all other dark-spored agarics by having either Coprinus-type parallel sided gills or a cellular cap cuticle. The family is composed of three sub-families, the Coprinoideae, the Psathyrelloideae, and the Panaeoloidea which between

them include five genera, Coprinus, Psathyrella, Lacrymaria, Panaeolus, and Panaeolina. "The sub-families and genera are natural units" (Singer, 1962) and this probably explains why it is relatively easy to identify a specimen first to its family and then to its genus. Good keys to families and genera of agarics are given by Henderson, Orton and Watling (1969).

Some photographs of Psathyrella fruit-bodies are shown in Chapter 10. The following generic description has been drawn up from a combination of the lengthy description of Singer (1962) and the more popular one of Wakefield and Dennis (1951). Some of the terms are illustrated in Figs. 1.1-9.

> "Cap convex or gibbous, often hygrophanous when dry; cap cuticle cellular; veil present or absent; stipe central, often covered by a fibrillose veil; gills more or less adnate; gill trama regular to sub-regular, pigmented or hyaline; basidia usually 4-spored; spores black or brown with germ pore, elliptical; cystidia usually present on gill surfaces and margins."

A history of the generic limits is summarised by Smith (1972) in the introduction to his book. The species now included in Psathyrella were originally scattered through the different groups of Agaricus in the sense of Fries (1838). These species were combined into a single group called Drosophila by Quèlet (1886). The groups of Agaricus were raised to generic rank by Saccardo (1887). Quèlet's concept and name were adopted by Romagnesi (1939) and Kühner and Romagnesi (1953). However, the name Psathyrella (Fr.) Quèlet (1872) predates the name Drosophila Quèlet (1886) and so fortunately Psathyrella is now considered to be the valid name for



Explanations of some terms often used in Psathyrella taxonomy

Fig. 1.1 Cap (convex)

Fig. 1.2 Cap (gibbous)

Fig. 1.3 L.S. of cap showing adnate gills

Fig. 1.4 Veil cells

Fig. 1.5 Cellular cap cuticle

Fig. 1.6 Regular gill trama

Fig. 1.7 Basidia

Fig. 1.8 Elliptical basidiospores

Fig. 1.9 Cystidia

the genus.

Within Psathyrella the sub-generic classification of Singer (1962) is based on the Friesian groups of Lacrymaria, Psathyra, Psathyrella and Hypholoma in addition to Homophron and two totally North American sub-genera Conocybella and Cystopsathyra. Smith (1972) revised Singer's classification and divided the genus into eleven sub-genera including Lacrymaria and Panaeolina. These latter two are considered to be separate genera by the British taxonomists (Dennis, Orton and Hora, 1960).

It has already been mentioned that it is relatively easy to identify collections to the generic level, but the difficulties in identification are overwhelming at the level of the species. About 60 species are included in the British flora by Dennis, Orton and Hora (1960), and over 400 species are recognised by Smith in North America. A few keys to the European species are available but not one of them seems to be satisfactory. The key of Lange (1939) includes only about a third of the British species, though it is clear and easy to use. There are also two descriptive keys, one of Moser (1955) in German, and one of Kühner and Romagnesi (1953) in French. They contain most of the European species but are clumsy and imprecise. Like all descriptive keys they attempt to avoid detailed description and so provide neither a usable means of identification nor a list of species descriptions. An example of a typical Kühner and Romagnesi dichotomy is translated below to show the sort of difficulties which are encountered.

> "Cap particularly brightly coloured, at first dark fawn brown but warm, becoming chocolate brown and keeping its ochraceous shade, when dry alutaceous-ochraceous, but warm,

with a faint fleshy hue which is not predominant, very micaceous. Stipe of young specimens fawn-ochraceous, more intense than in other species, then paling starting from the top. Trama compact, coloured bright yellow brown (sometimes extraordinarily intensely in young specimens). Spores 12-15.5 x 5.5-7.2 µm in four spored races. Grows in shady places.....P.picta Romagn.

Cap, stipe and trama less strongly coloured..... to the next dichotomy.

Psathyrella fruit-bodies are simply constructed and offer few quantitative or easily expressed characters. An assessment of the morphological characters of the fruit-body has been made by Romagnesi (1939). He concluded that the only characters which could be used reliably in a classification of the genus are colour and dimension of the spores, shape and position of cystidia and the presence of yellow pigment in the cuticle and trama.

In spite of this hundreds of species have been described using morphological criteria alone. The large number of species is the result of the adoption of a narrow morphological species concept in an attempt to define reasonably discrete units in an apparent continuum. This situation is perhaps extreme in Psathyrella but is not uncommon in other genera. Smith (1968) considers the narrow species concept to be the only one "with real meaning" in the higher fungi in general.

The question of what constitutes a unit of real meaning in taxonomy has been extensively discussed but evidence of breeding behaviour is too rarely included. Several factors are responsible for

this traditional dependence on the morphological characters of the fruit body to the exclusion of mycelial characters and breeding evidence. Agaric taxonomists are using the same methods as their higher plant counterparts but are many years behind them. Agaric taxonomy is probably still at the pioneer or consolidatory phase and perhaps the biosystematic phase is still to come. On the other hand fungi are sufficiently unlike higher plants to require an approach of their own. Who for example would consider classifying bacteria on morphological grounds alone? The traditional dependence on the morphological characters of the fruit body is also encouraged by the amateurs whose contribution to agaric taxonomy is considerable. They have no facilities for sterile culture work and are often ignorant of the more theoretical aspects of fungal biology. Even Professor Smith, who is no amateur, is scornful of breeding evidence and in his own laboratory treats all his specimens with preservative and so they can never be grown.

The first requirement for a better understanding of the processes of evolution and of the factors responsible for the existing taxonomic confusion is complete familiarity with the life cycle. The life cycle of a typical Psathyrella is shown in Figure 1.10. The important aspects of the life cycle will be described in detail so as to make clear certain points and to introduce some definitions.

The basidiospores germinate to give monokaryotic hyphae in which each cell has one or several nuclei of a single genotype. By vegetative growth a branched, septate mat of hyphae or mycelium is formed. Asexual spores or oidia are sometimes formed on this mycelium on short branches or oidiophores. Most basidiomycetes are heterothallic, that is the

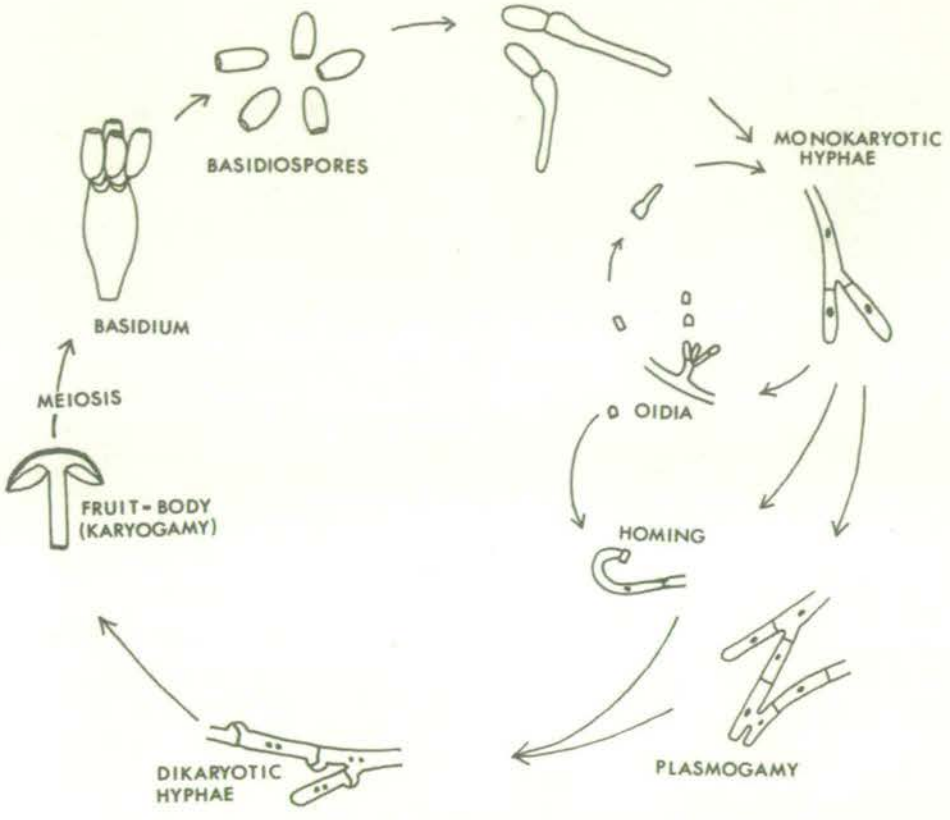


Fig. 1.10 Life cycle of a typical Psathyrella

monokaryotic mycelium does not form fruit-bodies until it becomes dikaryotised by a nucleus of a different genotype. The two types of nuclei required for sexual reproduction are not borne on special, morphologically distinct gametangia. The nuclei meet following the fusion of hyphae from two different mycelia or following the fusion of hyphae with oidia. Fusion of cells (plasmogamy) is not followed by the fusion of nuclei (karyogamy). The two nuclei coexist unfused in the single dikaryotic cell. Reciprocal dikaryotisation of the resident mycelia takes place only if the nuclei are compatible and if both belong to the same breeding group. The compatible nucleus migrates through the cells of the resident mycelium, dividing by mitosis in some of the cells on the way. The regular conjugate division of the two nuclei in a dikaryotic cell occurs by the formation of clamp connections at each septum. Clamp connections are characteristic of most basidiomycete cells though it seems that they are not essential for the occurrence of regular conjugate division. In a few species of Coprinus, for example, clamp connections are never formed.

This sequence of events in the basidiomycete life cycle has long been known. The milestones in the developmental history are described by Raper (1966) in a survey of fungal incompatibility. The first major discovery was that of clamp connections by Hoffman in 1856. Heterothallism was first discovered by Blakeslee (1904) in the Zygomycetes. The importanee of the temporal separation of plasmogamy and karyogamy was recognised by Raciborski (1896). Thus the existence of the dikaryotic phase became known. From then the pioneer work on incompatibility was done by Bensaude (1918) and Kniep (1920). Bensaude first recognised the association of nuclei in conjugate pairs and discovered heterothallism in the basidiomycetes. A few years later Kniep (1920) unaware of

Bensaude's work, rediscovered heterothallism, recognised the complexity of mating patterns, and in a series of papers founded a possible genetic basis for the incompatibility system.

Fruit-body primordia are usually formed only on dikaryotic, clamp-bearing mycelia though exceptions to this rule are known. The primordia develop by rapid division of lateral branches to form a tight cluster of hyphae. The innermost cells of the cluster become compacted to form a pseudoparenchymatous mass of tissue. When the cap and stipe expand the outer envelope of the primordium, (or veil) is ruptured. Remnants of the veil are sometimes found on the cap and stipe and are considered to be an important taxonomic character. The gills bearing basidia and cystidia are formed on the underside of the cap. Fusion of the two nuclei of the dikaryon to form a diploid nucleus takes place in the young basidia. Meiosis occurs soon after, and the four meiotic products become the nuclei of the developing spore tetrad. The mature basidiospores have two nuclei which are formed by a mitotic division. The basidiospores are shed soon after the expansion of the cap and stipe. When they fall onto a suitable substrate they germinate and the life cycle is repeated. Two to three weeks is the average length of a Psathyrella life cycle in the laboratory. It is therefore possible for the life cycle to be repeated several times in a single season.

In a group with such a versatile life cycle it would not be surprising to find rapid evolution of genetically isolated breeding groups. A newly isolated population will probably retain the morphological features of its ancestors, but with time differences in fruit-body morphology may accumulate and the breeding groups may then become

recognised as separate morphological species. However, the taxonomist's judgement is always subjective; an objective measure of the discontinuities can be found by studying the breeding biology.

Mycelia can be grown in culture by the germination of basidiospores. They offer a few additional morphological chafacters such as colour of mycelium, diameter of hyphae, morphology of oidia and oidiophores. But the main reason for growing mycelia in culture is so that their reactions to each other can be studied. The reactions which occur when mycelia meet may give an indication of the relationship of the original isolates. If the monokaryotic hyphae which meet fuse with one another and form dikaryons then there is little doubt that the mycelia belong to the same breeding group. If no dikaryons are formed then different breeding groups may be involved. Sterility between mycelia of different breeding groups can sometimes be confused with incompatibility between mycelia of the same mating type, but if geographically distant isolates are involved it is unlikely that they will have mating types in common. However, in order to be certain of distinguishing sterility from incompatibility, several monokaryons of each isolate have to be used in each test of crossability.

Mycelial crossing is simple to test. Two mycelia are inoculated on an agar plate and when the colonies meet the formation of dikaryotic hyphae is noted. Usually mycelium from the junction line between the colonies is examined and the dikaryotic hyphae, if present, are distinguished by the clamp connections. This method was used by Biggs (1937) in Corticium coronilla, Mounce and Macrae (1936, 1937, 1938) in several groups of the Polyporaceae, Lange (1952) in the genus Coprinus, Burnett and Boulter (1963) in Mycocalia denudata, and by several other authors in many other groups of fungi. Adams and Roth (1967) used aversion reactions to "detect genetically distinct mycelia". A positive

aversion reaction can usually be seen macroscopically as a line of tangled hyphae at the junction line between the mycelia. Unfortunately a negative result gives no measure of similarity.

The reactions which follow the meeting of hyphal cells can also be observed microscopically. This may be necessary particularly in those groups of fungi in which clamp connections are not formed. Direct observation of hyphal anastomosis was first used by Buller (1924) in Coprinus and was also used by Davidson, Dowding and Buller (1932) in their studies on the dermatophytes in the Fungi Imperfecti. Vandendries and Brodie (1933) considered the test of hyphal anastomosis to be more convenient than those based on crossing and clamp formation. They used it to study relationships in many groups of the fungi, including Psathyrella. However, others have found considerable difficulties in applying the test in other groups.

Another test which uses microscopical observations is the homing of hyphae towards oidia, first observed by Kemp (1970) and soon afterwards by Bistis (1970). The homing reaction is so named because the tips of the hyphal cells grow towards and fuse with the oidia. Homing is easier to detect than hyphal anastomosis for the directed growth of hyphal tips towards the oidia can be seen under the low power objective of a microscope and the whole test can take less than one hour.

Thus the formation of clamp connections, aversion reactions, hyphal anastomosis and the homing of hyphae towards oidia have all been used to test the affinity of isolates. The occurrence of clamp connections is an indication of plasmogamy and the formation of dikaryons, and is therefore the most objective of these tests for the delimitation of breeding

groups. F_1 fruit bodies should ideally follow but since only a small percentage of basidiomycetes have been successfully fruited in culture, it is not practicable to wait for their formation before defining the breeding groups.

Once the breeding groups are objectively defined it is possible to compare them with the 'species' established on differences in fruit-body morphology. Furthermore, studies on the variation of morphological characters within and between the breeding groups can provide a means for the evaluation of the predictive value of those characters on which taxonomic units are usually based.

The following plan of study was adopted. An attempt was made to establish cultures of all isolates that were collected. Isolates were divided into groups on the basis of morphology. Three morphological complexes were chosen for further investigation. The cultures were examined for new morphological characters. The patterns of incompatibility were studied in a number of selected isolates using mycelial mating tests. The structure and function of oidia were investigated. The study of oidial morphology and surface structure was made in an attempt to resolve the physiological basis for the recognition of oidia by hyphal cells. The homing reaction and the events which follow it were given particular attention. In each of the morphological complexes mycelia were crossed in order to establish breeding groups. The morphological discontinuities between the breeding groups were investigated. The constancy of some morphological characters was evaluated using comparisons within and between the groups.

In order to avoid confusion it is important to use a strictly

defined terminology. Discussions on the species problem are often circular because the term 'species' tends to be used loosely before it is fully defined. The terms 'species' and also 'individual' will therefore be avoided in this thesis until the final discussion. Some of the other terms used are defined below.

isolate:	- a single fruit-body collected from the wild and the mycelial progeny directly derived from it.
monokaryon:	- monokaryotic mycelium
dikaryon:	- dikaryotic mycelium
clamp:	- clamp connection
homing:	- homing of hyphae towards oidia
mating test:	- a test between two monokaryons derived from the same isolate to determine mating type.
crossing test:	- a test between two monokaryons derived from different isolates to determine breeding groups.
incompatibility:	- failure to mate due to similarity of mating type.
sterility:	- failure to cross
incompatibility system:	- pattern of mating between monokaryons of the same isolate.
breeding system:	- the organisation of plasmogamy and karyogamy in a breeding group which determines the degrees of similarity or difference

between the gametes. (modified after Darlington and Mather, 1949).

breeding group: - a group of isolates between which successful crossing takes place

In defining these terms some of the basic problems of the thesis are indicated.

CHAPTER 2 : COLLECTION OF SPECIMENS AND GENERAL CULTURE TECHNIQUES

2.1. Collection and examination of specimens

Almost 100 toadstools of the genus Psathyrella were collected in Britain from their natural habitats during three collecting seasons. Each freshly collected specimen was brought back to the laboratory and described. For each isolate examined the characters listed below were scored and recorded.

Macroscopic characters:

Cap: size, shape, colour, texture, veil, margin.

Gill: insertion, colour, breadth, edge.

Stipe:length, breadth, colour, veil, base.

Spore deposit colour.

Microscopic characters:

Spores: number per basidium, approximate size, shape.

Facial cystidia (pleurocystidia): approximate frequency, size, shape.

Cystidia at gill edge (cheilocystidia): approximate frequency, size, shape.

Cystidia on stipe (pileocystidia): approximate frequency, size, shape.

Basidia: approximate size, shape.

Gill trama: colour, arrangement.

Cuticle cells: approximate size.

Veil cells: presence of clamp connections, approximate size, shape.

Most descriptions were illustrated with water colour drawings or colour photographs. The characters most vulnerable to change were

recorded first. The techniques used for the preparation of fresh specimens for description were mostly simple but many useful hints were learned from Watling (1968). Spore prints were made on glass slides according to the instructions of Henderson, Orton and Watling (1969).

The specimens were dried and stored in separate containers containing blue crystals of self indicating silica gel. The advantages of drying fungal specimens in silica gel are discussed by Hosêney (1963). Fresh silica gel was used for each specimen to prevent cross contamination with foreign spores. Detailed descriptions of the herbarium material were made for a few critical specimens. The method followed for the examination of herbarium material was that of van Waveren (1968).

Early on in the investigation attempts were made to identify each specimen to the species using the published keys, but it soon became evident that a meaningful identification was usually not possible. From then on each specimen was numbered and asigned to a broad morphological group.

In addition to the Psathyrella isolates personally collected in this country, some were obtained from named herbarium packets deposited in the Herbarium of the Royal Botanic Garden, Edinburgh. A few more were sent by other mycologists.

2.2. Establishment of a culture collection

Initially an attempt was made to set up cultures of all the available Psathyrella isolates, but with time most of the experimental work became limited to three sections of the genus. Mycelia of about 100 isolates were grown in culture during the course of the investigation.

All cultures derived the same fruit body were given the same code number (e.g.21). Each monokaryon was further distinguished as 21.1, 21.2, etc. Most of the cultures were obtained by the germination of basidiospores. In a few cases dikaryotic mycelia were grown by culturing the tissues of fresh fruit bodies. A list of all the isolates cultured, and also those in which attempts at culturing failed, are listed in Appendix I. A list of cultures placed in recognised culture collections is given in Appendix II.

2.3. Media

The basidiospores were germinated and mycelia grown on agar in sterile disposable plastic petri dishes. Two media were used almost exclusively throughout. Horse dung extract medium was used for the growth of all coprophilous and P.candolleana isolates. Malt agar was initially used for most of the soil-growing isolates, but later horse dung extract medium was found to be better. The compositions of the two media are given below.

Dung Extract Medium (Lange 1952)

Oxoid Agar No.3	12 g
Maltose	5 g
Magnesium sulphate	0.5 g
Calcium nitrate	0.5 g
Potassium dihydrogen phosphate	0.25 g
Oxoid Mycological Peptone	0.1 g
Tap Water	900 ml
Horse Dung Extract	100 ml

Malt Extract

Oxoid Agar No.3	12 g
Oxoid Malt Extract	16 g
Oxoid Mycological Peptone	0.1 g
Tap Water	1000 ml

Different media were used for fruit body production in some isolates. These are described in section 10.4. All glassware and media were sterilised at a pressure of 15 lb per sq. inch for 15 minutes.

2.4. Plating of basidiospores

Monokaryotic mycelia were needed for all the mating type and crossability experiments. Most were obtained by the isolation of colonies derived from single randomly sown basidiospores. When only dikaryotic mycelia were isolated, monokaryotic mycelia were obtained from the dikaryotic by maceration.

The most reliable techniques for isolating colonies derived from single basidiospores is to isolate ungerminated basidiospores by micromanipulation. The technique is however clearly frustrating if spore germination percentage is low. A more practical though less accurate method was therefore used.

The basidiospores were mostly obtained from gills or spore prints of dried specimens. Watling (1963) found that spores from herbarium material of the family Bolbitiaceae germinate better if incubated in a water-saturated atmosphere overnight. All the spores to be germinated were therefore treated in this way before plating. A scrape of spores from a spore print or a small section of a gill was transferred onto a sterile slide and the slide supported by lengths of glass rod over some sterile water in a petri dish. The spores were left to incubate at room temperature for 12-30 hours. Spores were then transferred on a tungsten loop into McCartney bijoux bottles half filled with sterile water. Two loopfuls of spores in approximately 3 ml of water usually provided an adequate

concentration. The spore suspension was mixed by sucking in and out with a Pasteur pipette and approximately 0.1 ml of the suspension was then plated out onto the agar surfaces. The droplets of spore suspension were spread evenly with a glass spreader and the plates incubated at 24°C. The spore suspension remaining in the McCartney bijoux bottles was given heat treatment in a 'hot box' at 45-50°C for 60-90 minutes. 0.1 ml aliquots of the suspension were then withdrawn and spread as before.

The germination conditions were selected only after a number of different techniques had been tried. The experiments which were carried out will be described in detail in the next chapter.

After about 48 hours incubation the plated basidiospores germinated to give barely visible colonies. The agar surface was scanned and each clearly separate colony was 'ringed' using the flamed base of a Pasteur pipette. The 'ringed' colonies were examined under the low power objective of the microscope. Those that clearly originated from the germination of a single basidiospore were isolated and subcultured.

2.5. Isolation of dikaryotic mycelia from the tissues of fresh fruit bodies.

Most cultures were established by the germination of basidiospores as described above but in some cases dikaryotic mycelia were isolated directly from the tissues of fresh fruit bodies. Small pieces of gill, stipe and pileus trama were carefully dissected using sterile instruments and placed on the underside of the lid of an agar plate. The agar layer was then cut round

the periphery with a flamed metal spatula and inverted onto the lid. Some dikaryotic hyphae normally grew from the tissues through the agar, leaving the bacterial contaminants behind. The dikaryotic hyphae were subcultured when they reached the agar surface.

2.6. Maceration

Monokaryotic components could be recovered from the dikaryotic cultures by maceration in a Jencons Tube Tissue Grinder (Type MTG 3/P*). The tube and plunger were sterilised by rinsing in alcohol and were flamed carefully. 4 ml of sterile water was poured into the tube and a small lump of hyphae and agar approximately 2 mm^3 was placed on top of the plunger. The tissue was macerated gently using about 8 movements of the plunger. 0.1 ml drops of the suspension were plated onto the surface of the agar plates and spread evenly using a glass spreader. After incubation at 24°C for about 3 days it was usually possible to identify a few compact, slow growing monokaryotic colonies among numerous dikaryotic colonies. The small colonies were subcultured and checked for the absence of clamp connections.

2.7. Maintenance of stocks

About 15 monokaryotic mycelia and one or two dikaryotic mycelia of each isolate were stocked on agar slopes in bijoux vials. The stocks were stored in the cold room at 4°C and subcultured every 4-6 months. A tungsten needle sharpened in sodium nitrite was used in subculturing.

For shorter term storage the liquid paraffin method was used. A perforated disc of perspex was sterilised with alcohol or with U.V. and was lowered into a sterile petri dish containing

sufficient paraffin oil to fill the holes without flooding the surface of the disc. The perforations in the disc were cone shaped, 3 mm in diameter, on the upper surface, and about 1 mm on the lower surface of the disc. Each oil filled cone was used to house an inoculum of a single stock culture. There were 7 rows of 7 cones per disc.

This simple apparatus was designed by Kemp (unpublished). The method has two main advantages over storage on agar slopes. Firstly there is a considerable saving of space. Secondly, since the inocula are effectively dormant, the opportunities for the occurrence and propagation of mutations are eliminated. The percentage recovery of Psathyrella stocks after 5 months of storage under paraffin oil was recently proved to be 95%, suggesting that the method could be used for the routine storage of stocks.

CHAPTER 3 : BASIDIOSPORE GERMINATION

3.1 Introduction

The technique of plating imbibed but otherwise untreated spores which was used at the beginning of the study gave successful germination in only about a third of the isolates. But even in the successful isolates the percentage germination was low. Other experimental conditions which favour germination were therefore investigated.

The effects of heat, cold and furfuraldehyde were investigated since they have been found to stimulate spore germination in other genera. Heat is known to have been successful in activating spore germination in Neurospora tetrasperma (Goddard, 1935) and in the basidiomycete Cyathus stercoreus (Brodie, 1948). Heat pretreatment was also found to sensitise the ascospores of Neurospora tetrasperma to furfuraldehyde (Sussman, 1954). Cold storage was used by Whitney (1966) for the spores of Polyporus tomentosus.

3.2 Materials and Methods

The main experiment was carried out using basidiospores of Isolate 17. (P.gracilis, Breeding Group II ≈ P.microrrhiza). The effect of heat alone, was also investigated in several other isolates.

Small pieces of gill of Isolate 17 were imbibed in a water-saturated atmosphere for 15 hours as described in section 2.4. A schematic representation of the treatments then used is given in Fig.3.1. The following summary includes the abbreviations used in Fig. 3.1 and Table 3.1.

H - heat treatment for 1¼ hours at 46°C

h - no heat treatment

C - cold treatment at 4°C for 24 hours

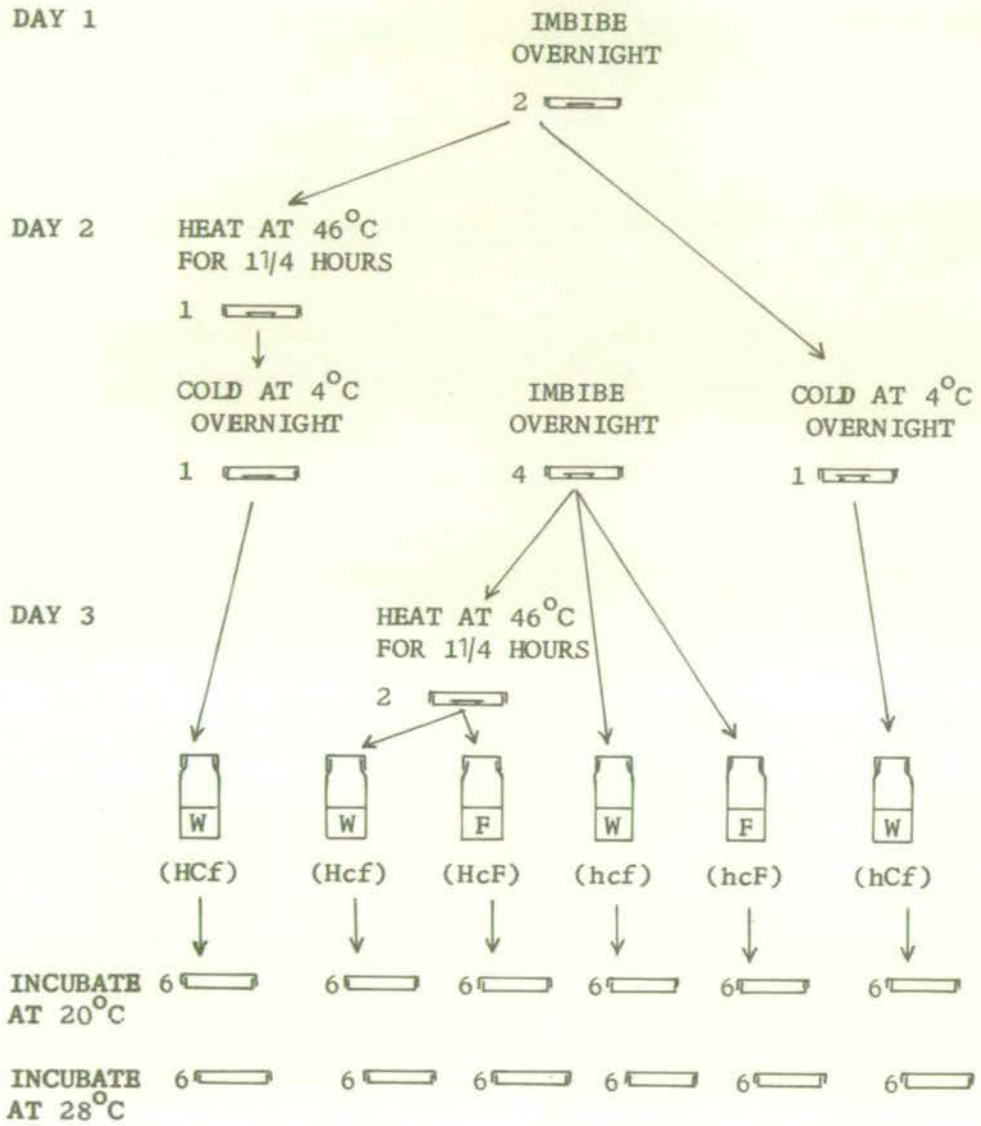


Fig.3.1. A schematic representation of the treatments used in the spore germination experiment. (Abbreviations as in Section 3.2).

c - no cold treatment

F - wash in furfuraldehyde (1×10^{-3}M) for 60 mins.

f - wash in water for 60 mins.

The hot or cold treatments were carried out while the spores were still on the slides. Approximately two loopfulls of spores were then introduced into the bijoux bottles each containing 3 ml of furfuraldehyde or sterile water. The furfuraldehyde was not autoclaved but was sterilised with chloroform.

The concentration of spores in suspension in the bijoux bottles was estimated using a haemocytometer. 0.1 ml samples of the spore suspension were pipetted onto the agar surface and were spread using a glass spreader. The plates were incubated in the dark at 20°C and 28°C. There were thus twelve treatments and each was replicated six times. The number of colonies growing on each plate was counted after 5 days' incubation.

3.3 Results

The results of the experiment are given in Table 3.1.

It is clear from the table that heat treatment caused almost a tenfold increase in spore germination. It is not necessary to carry out any statistical tests to show that this is a very significant increase. Furfuraldehyde by itself increased the percentage germination only slightly, while the effect of heat and furfuraldehyde combined, did not appear to be any more favourable than that of heat alone. Cold treatment, on the other hand, appeared to have a distinctly inhibitive effect. More colonies were formed after incubation at 28°C than at 20°C, though in a subsequent experiment it was found that growth rate following germination was better

Table 3.1. The effect of 12 different treatments on basidiospore germination in isolate 17. (* = contaminated plate; other symbols are explained in section 3.2).

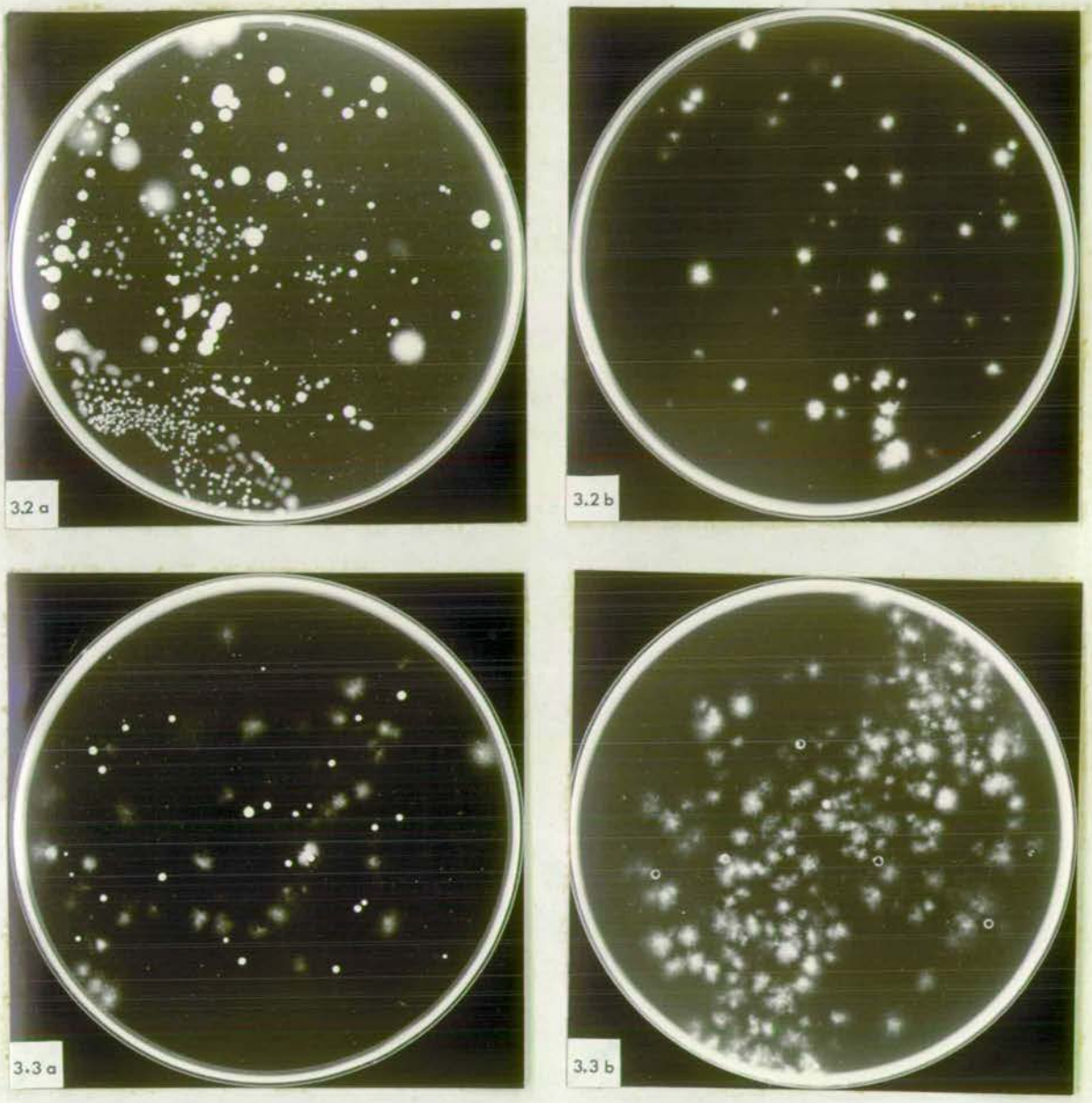
Treatments	Hcf		HcF		HCf		hcf		hcF		hCf	
Incubation temperature	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
Approximate number of colonies per plate in each of the six replicates	200	200	200	200	75	100	18	34	40	40	8*	*
	200	200	100	200	100	100	12*	20	50	50	11*	9
	200	200	200	200	100	75	18	17	40	*	7*	5
	100	200	200	200	75	100	22	30	50	100	10	10
	200	250	200	200	100	50	31	50	55	100	7	11
	200	300	150	250	50	75	50	40	55	*	12	10
Approximate number of spores plated	2100	2100	1900	1900	1750	1750	1900	1900	2950	2950	1200	1200
Average % germination	8.7	10.7	9.2	11.0	4.8	4.8	1.2	3.2	1.6	2.5	0.8	0.8

at the latter temperature. Another favourable effect of heat treatment was a marked reduction in the number of contaminants. In this particular experiment the isolate was only slightly contaminated and so the effect of heat treatment in inhibiting the growth of contaminants was not so obvious. The increase in the number of colonies and the reduction in the number of contaminants can be seen clearly from the contact photographs shown in Figs. 3.2 and 3.3. In these, two other isolates from the P.gracilis complex were used.

Heat treatment was easy to use and so became part of the routine procedure described in Section 2.4. Each time untreated spores were also plated for control. The technique was modified slightly in that heat was given to spores only after they had been suspended in sterile water. This gave just as good results and simplified the procedure. Incubation was usually carried out at 24°C.

In this way the effect of heat was tested on spores of about twenty isolates belonging to different breeding groups. Usually there was a marked increase in the number of colonies per plate. In a few cases there was no difference between the treated and untreated plates. In no case was the treatment found to have an inhibitive effect on germination.

It was noticed that the ease with which spores germinate in culture is dependent on the original habitat of the isolate. All the coprophilous isolates germinated as well without heat treatment as after it. Some of them germinated readily even in a thin film of distilled water. However, in most isolates from wooded or roadside habitats, a marked increase in spore germination following heat treat-



Effect of heat pretreatment on basidiospore germination in isolates of the P.gracilis complex showing increased colony formation and decreased contamination.

Fig. 3.2 (a) Basidiospore plating without heat pretreatment, Isolate 101.

(b) Basidiospore plating after pretreatment for 1 hour at 50°C. The same spore suspension was used as in (a).

Fig. 3.3 (a) Basidiospore plating without heat pretreatment, Isolate 111.

(b) Basidiospore plating after heat pretreatment for 1 hour at 50°C. The same spore suspension was used as in (a).

ment was found. In each case the number of contaminants was reduced and so it was easier to isolate the mycelial colonies. Isolates from coprophilous habitats not only germinated more readily, but also retained their viability longer. For example, the spores of P.coprophila (Breeding Group I) germinated successfully even after 47 months of storage in the herbarium. Other spores lost their viability relatively quickly. About 90% of the freshly shed spores of P.candolleana germinated, but none was viable after 15 months of storage. No evidence was found in any of the isolates sampled for the existance of a dormant period. All spores germinated best when fresh and their viability declined steadily with time. The absence of a dormant period in the basidiospores of other genera was also observed by Fries (1943).

Spore germination was usually found to be independent of spore density except for the spores of P.conopilea (Isolates 93 and 107) which germinated only when sown very densely. Quintanilha (1941) made a similar observation in P.subatrata. It was interesting to find that in the British checklist (Dennis, Orton and Hora, 1960) P.conopilea (Fr.) Pearson and Dennis and P.subatrata (Batsch ex Fr.) Gillet are considered to be synonymous.

3.4 Discussion

Adequate germination is necessary in order to obtain a sufficient number of monokaryons of each isolate in culture for use in the mating and crossing tests. Conditions that improve germination have therefore been investigated. The effect of heat treatment in improving spore germination is physiologically difficult to explain,

especially as temperatures of 50°C are not commonly experienced by spores in their natural habitats. However, since the physiological aspects of spore germination are outside the scope of this thesis this topic was not explored further.

CHAPTER 4 : INCOMPATIBILITY SYSTEMS

4.1 Introduction

It is important to be familiar with the pattern of matings between monokaryons derived from a single isolate before investigating the crossability of different isolates.

An account of the incompatibility literature is first given. The method of determining the incompatibility systems is then developed using two isolates as examples. In the third section the incompatibility systems of several isolates are described. In a few isolates modifications from the basic patterns of incompatibility were found. Some of these are described in the fourth section of the chapter. One of the isolates was found to have a particularly interesting incompatibility system based on three loci. This system was examined in some detail and the experiments which were carried out are described separately in Chapter 5.

In heterothallic basidiomycetes incompatibility is determined by one or two unlinked incompatibility factors. The term factor is used rather than locus as each factor consists of at least two subunits which may not always be closely linked (Papazian, 1950; Takemaru, 1957). When a single factor is involved it is designated A, and when two factors are involved they are designated A and B. Each subunit of the A and B factors consists of a multiple allelic series. Heterozygosity for the incompatibility factors is necessary for the mating to result in the completion of the life cycle. The system determined by a single incompatibility factor is sometimes termed bipolar following the initial use of the term by Burgeff (1920) in Ustilago violacea. The mating system which is determined by two incompatibility factors is

sometimes termed tetrapolar following its first use by Bauch (1930) in Ustilago longissima. The terms bipolar and tetrapolar refer to the mating types of the meiotic products. The terms unifactorial and bifactorial indicate the genetic basis of the system and are now more often used.

Basidiospores obtained from the same fruit-body are of two mating types in a unifactorial system and of four mating types in a bifactorial system. Each monokaryon can mate with half of the sister monokaryons in a unifactorial system, but only with a quarter of the sister monokaryons in a bifactorial system. In both systems mating between non-sister monokaryons, derived from unrelated fruit-bodies of the same breeding group, is virtually unrestricted because of the many alternative alleles at both incompatibility loci.

When two monokaryotic mycelia meet the fusion of hyphae takes place irrespective of mating type. However, the sequence of events which follows depends on the mating type of the two nuclei. If the nuclei are heterozygous at each incompatibility locus the new compatible nucleus migrates through the cells of the resident monokaryotic mycelium dividing by mitosis in some of the cells on the way. The regular conjugate division of the two nuclei occurs by the formation of clamp connections at each septum. The dikaryotic mycelium is usually more vigorous than the parent monokaryons. The sexual process is completed when fertile fruit-bodies are formed in a suitable environment.

In a bifactorial system the functions of the A and B factors have been determined by observing the reactions of matings which are heterozygous for one factor. It was found that heterozygosity at the A

factor is responsible for most of the stages of clamp formation, and that heterozygosity for the B factor alone is sufficient to allow nuclear migration (Fulton, 1950). As heterozygosity for both factors is necessary for the completion of the sexual process the incompatibility mechanism is probably based on complementation.

Incompatibility systems are investigated by mating a randomly selected sample of monokaryotic mycelia in all combinations, and scoring the matings for the presence of clamp connections. Between 1920 and 1950 the incompatibility systems of many basidiomycetes were investigated in this way by many authors. The results for more than 350 species are summarised in list form by Whitehouse (1949) and Quintanilha and Pinto-Lopes (1950). However the methods used to determine the incompatibility systems have received little attention. The observations of Aschan (1954) were important because they drew attention to the method of mating type determination, and pointed out possible inconsistencies in previously published results.

When two compatible matings come in contact both are usually dikaryotised by nuclear migration. The extent of dikaryotisation is indicated by the presence of clamp connections. Mycelia which are heterozygous for only the A factor can be isolated from the junction line and these have false clamps (pseudoclamps) at most septa. In many species it is difficult to distinguish between true clamps and pseudoclamps. When testing for mating types it is therefore necessary to obtain information on nuclear migration as well as on clamps. Aschan (1954) found in Collybia (=Flammulina) velutipes that if only the junction line between the mated mycelia was examined for the presence of clamp

connections, pseudoclamps were mistaken for true clamps and so the bifactorial system was overlooked. If, however, mycelia from either side of the junction line were examined as well, the clamp forming matings could be put into two groups, those with clamps at the junction line only and those with clamps on both sides. In this way it was possible to distinguish four, not two mating types.

Several cases are known where bipolar and tetrapolar systems have been found for the same species by different authors. Some of these are listed in Table 4.1.

Table 4.1 Examples of species in which incompatibility systems have been determined to be unifactorial and bifactorial by different authors.

Species	Unifactorial	Bifactorial
Psilocybe coprophila	Gilmore (1926)	Vandendries (1937)
Annelaria (*Panæolus*) *separatus*	Vandendries (1923)	Routien (1940)
Hypholoma fasciculare	Vandendries (1923)	Funke (1924)
Coprinus macrorrhizus (= *cinereus*)	Routien (1940)	Bensaude (1918)
C.sphaerosporus)	Dickson (1934)	
) (= *lagopides*)		
C.funariorum)		Quintanilha et al (1941)

Mistaken identification could account for some of the cases where uni- and bifactorial systems have been recorded in the same species, but this is not so likely with the species listed above. The first three species listed are known to be taxonomically well defined. Recent studies

in Coprinus section lanatuli, to which both C.cinereus and C.lagopides belong have shown all the described species as well as several new ones to be bifactorial (Kemp, personal communication).

It is likely that the use of an incomplete scoring procedure followed by the mis-identification of true and false clamps accounts for wrong determinations of incompatibility systems in some of these cases. The determination methods used by the authors listed above were therefore examined in detail. It was found that those authors who obtained bipolar results made no effort to detect nuclear migration, or else the details of the scoring procedure are omitted from their accounts.

Aschan's findings have also received little attention in the more recent literature. In several recently published papers compatible matings were distinguished by the presence of clamp connections at the junction line only (Watling, 1964; Neuhauser and Gilbertson, 1971; Butler, 1972). Ginns (1974) reinterpreted several examples of previously described unifactorial two-spored species to be bifactorial. Using mating tables of other authors he found that the failure rate in matings was less when the tables were rearranged according to the bifactorial pattern. However Ginns seemed not to be aware of the significance of the nuclear migration information, and he did not cite Aschan's important publication.

In the present study the importance of Aschan's findings was only realised towards the end of the research. Many mating type experiments had to be repeated, but the conclusions which were eventually made were the same as those of Aschan in Flammulina velutipes. The incompatibility systems of the two new 'species' F.coprophila and

P.fimetaria were initially wrongly determined (Watling and Jurand, 1971). The experiments were repeated and the systems reinterpreted to be bifactorial shortly after publication. A similar mistake was made by Macrae (1941) in Hirschioporus abietinus. Bipolarity was first recorded and it was not till many years later that Macrae (1967) reinterpreted and corrected her earlier results.

In the next section the mating type experiments carried out on P.coprophila and P.'fimetaria' before and after the publication of the paper are described and compared.

4.2 Development of a method for determining incompatibility using P.coprophila and P.'fimetaria'

4.2.1 Preparation of monokaryotic mycelia

Random samples of monokaryotic mycelia were obtained by germinating basidiospores from a single fruit-body. Tetrads were not isolated. In order for there to be a reasonable chance of obtaining at least one monokaryon of each mating type a certain minimum sample size of monokaryons had to be used. The probabilities were calculated using the following formula:

$$a_{ij} = (a_{(i-1)(j-1)} - a_{i(j-1)}) \frac{k-i+1}{k} + a_{i(j-1)}$$

in the formula: k = number of classes, e.g. 2 in a unifactorial system, 4 in a bifactorial system.

i = minimum number of classes appearing in the sample.

j = number in the sample.

The formula was used to draw up a table of probabilities (Table 4.2) from which the minimum sample sizes required for the mating

j	K = 4				K = 2	
	i=1	i=2	i=3	i=4	i=1	i=2
1	100.0	0.0	0.0	0.0	100.0	0.0
2	100.0	75.0	0.0	0.0	100.0	50.0
3	100.0	93.8	37.5	0.0	100.0	75.0
4	100.0	98.4	65.6	9.4	100.0	87.3
5	100.0	99.6	82.0	23.4	100.0	93.6
6	100.0	99.9	90.8	38.1	100.0	96.8
7	100.0	100.0	95.4	51.3	100.0	98.4
8	100.0	100.0	97.7	62.3	100.0	99.2
9	100.0	100.0	98.8	71.1	100.0	99.6
10	100.0	100.0	99.4	78.1	100.0	99.8
11	100.0	100.0	99.7	83.4	100.0	99.9
12	100.0	100.0	99.9	87.5	100.0	100.0
13	100.0	100.0	99.9	90.6	100.0	100.0
14	100.0	100.0	100.0	92.9	100.0	100.0
15	100.0	100.0	100.0	94.7	100.0	100.0
16	100.0	100.0	100.0	96.0	100.0	100.0
17	100.0	100.0	100.0	97.0	100.0	100.0
18	100.0	100.0	100.0	97.7	100.0	100.0
19	100.0	100.0	100.0	98.3	100.0	100.0
20	100.0	100.0	100.0	98.7	100.0	100.0
21	100.0	100.0	100.0	99.0	100.0	100.0
22	100.0	100.0	100.0	99.3	100.0	100.0
23	100.0	100.0	100.0	99.5	100.0	100.0
24	100.0	100.0	100.0	99.6	100.0	100.0
25	100.0	100.0	100.0	99.7	100.0	100.0

Table 4.2 Percentage probability of finding at least 1, 2, 3 or 4 classes and 1 or 2 classes (i) in samples of j monokaryons derived from the basidiospore progeny of bifactorial (K=4) and unifactorial (K=2) isolates.

type determination experiments were obtained. If the incompatibility system of an isolate was unknown, 15 monokaryons had to be isolated for there to be a 95% probability that the sample would contain at least one monokaryon of each mating type. For less critical mating type determinations it would be enough to sample 7 monokaryons, for if three mating types are found one could deduce that the mating system is bifactorial. Where the incompatibility system is suspected to be unifactorial it would be enough to sample 6 monokaryons. It has been assumed throughout the calculation that the mating type classes are equally distributed in the population of monokaryons.

4.2.2 Mating of monokaryons

Monokaryons derived from Isolate 84 (P.coprophila, Breeding Group I) and Isolate 59(P.'fimetaria' = P.coprophila, Breeding Group II) were used in this experiment. 15 monokaryons were mated in all combinations. The matings were made on horse dung extract agar, 4 per plate, placing the inocula about 4 mm apart. The inocula were incubated for several days at 24°C. The results were scored two or three days after the colonies had met.

4.2.3 Examination of mated mycelia

Method I

Initially the results were scored by examining the mycelium only at the junction line for the presence of clamp connections. The position from which samples were taken is indicated in Table 4.3. The mycelium and the underlying agar was first 'ringed' using the flamed base of a Pasteur pipette, and the samples were transferred with a needle to fresh agar plates. They were incubated for two days and examined

microscopically on the plate for the presence of clamp connections. If clamp connections were found the mating combination was scored positive. Differences in frequency were ignored.

Method II

The resident monokaryons as well as the junction line were examined for the presence of clamp connections. The occurrence of vigorously growing clamp-bearing hyphae at the peripheries of the mated colonies indicated that nuclear migration had taken place. The matings where nuclear migration appeared to be absent were then investigaged further. Five inocula were sampled from each mating (Table 4.3). They were transferred onto fresh agar, incubated and examined as those of Method I.

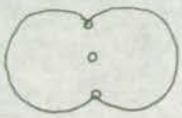
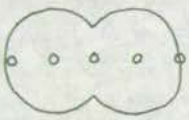
4.2.3 Results of scoring

The positions of the sample inocula, possible results and their interpretations are explained in Table 4.3. The original and rearranged results of mating 15 monokaryons in all combinations using Methods I and II are given in Fig. 4.1 (Isolate 84) and Fig.4.2(Isolate 59). In the rearranged tables the monokaryons having the same mating type are placed next to one another.

The results for these two isolates show that both clamp formation and nuclear migration have to be investigated in order to detect the bifactorial incompatibility system. It is difficult to distinguish true clamps from pseudoclamps by routine microscopical observations. Photographs of true and false clamps in Isolates 84 and 59 are shown on Figs. 4.3-4.6. The differences are slight and can only be distinguished with practice.

The same was found to be true for most of the other isolates studied. The mated mycelia were therefore examined according to Method II in all further determinations of incompatibility systems.

Table 4.3 Positions of samples taken from the mated mycelia, results and their interpretations. The notation used in the last column is that of Raper (1966).

Position of test samples	Result and Interpretation: clamps present • absent ○	nuclear migration	Symbol	Mating Types of mycelia
Method I 	• • •	?	+	A≠ e.g. A_1A_2
	○ ○ ○	?	-	A= e.g. A_1A_1
Method II 	• • • • •	bilateral	↙↗	A≠B≠ e.g. $A_1B_1A_2B_2$
	○ ○ • • •	unilateral	↗	A≠B≠ e.g. $A_1B_1A_2B_2$
	• • • ○ ○	unilateral	↙	A≠B≠ e.g. $A_1B_1A_2B_2$
	○ ○ • ○ ○	absent	∓	A≠B= e.g. $A_1B_1A_2B_1$
	○ ○ ○ ○ ○	absent	=	A=B≠ e.g. $A_1B_1A_1B_2$ or A=B= e.g. $A_1B_1A_1B_1$

4.3 Results of incompatibility systems of several different isolates

One or two isolates from each of the major breeding groups were selected for analysis. The breeding groups were first defined on the basis of crossing experiments which are described in Chapters 8,9 and 10. The mating tables are shown on Figs. 4.7-4.16.

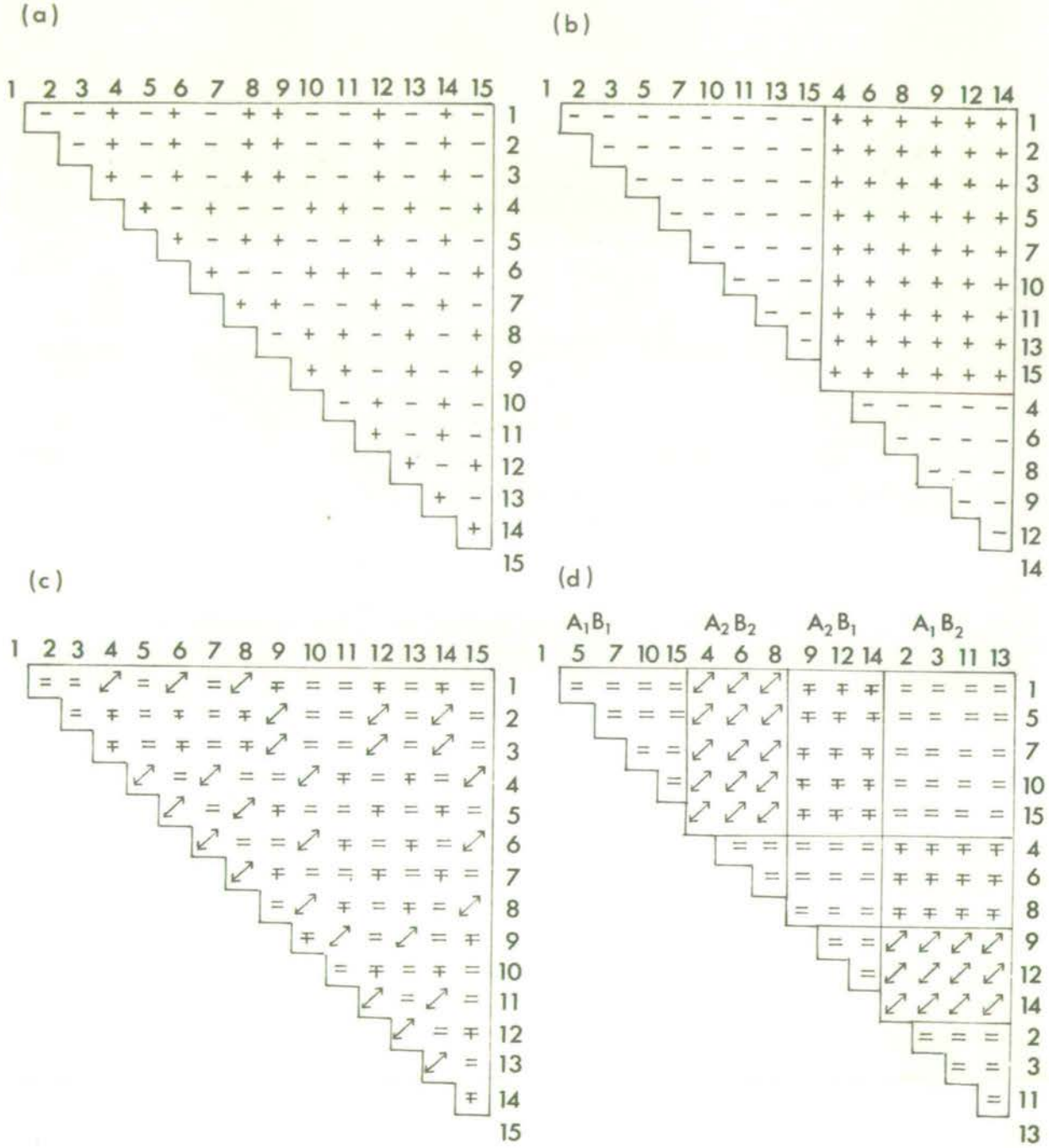


Fig. 4.1 Results of mating 15 monokaryons of Isolate 84 (P.coprophila Breeding Group I) in all combinations. (Symbols as in Table 4.3)

(a) Original mating table using Method I
(b) Rearranged mating table using Method I
(c) Original mating table using Method II
(d) Rearranged mating table using Method II

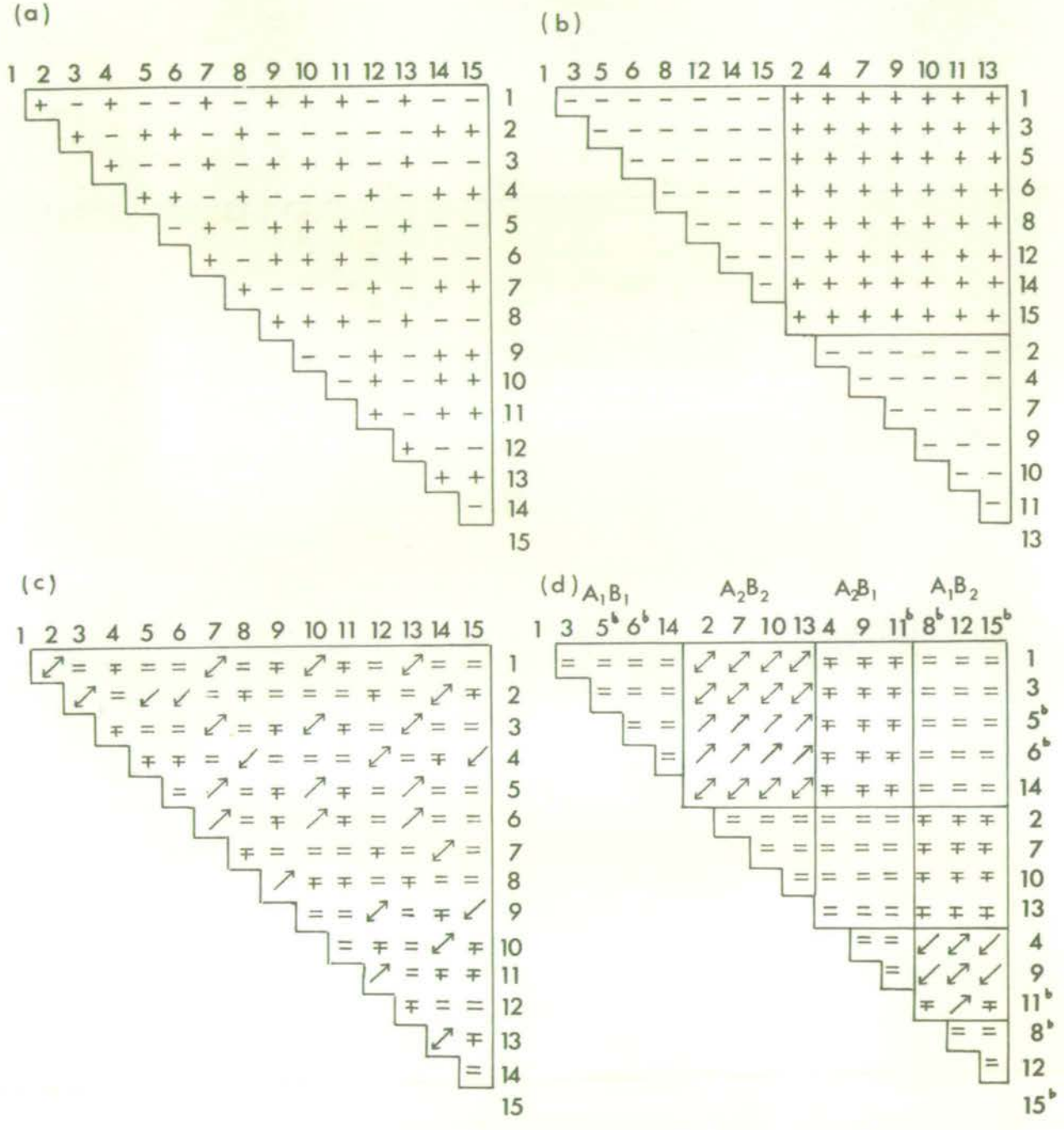
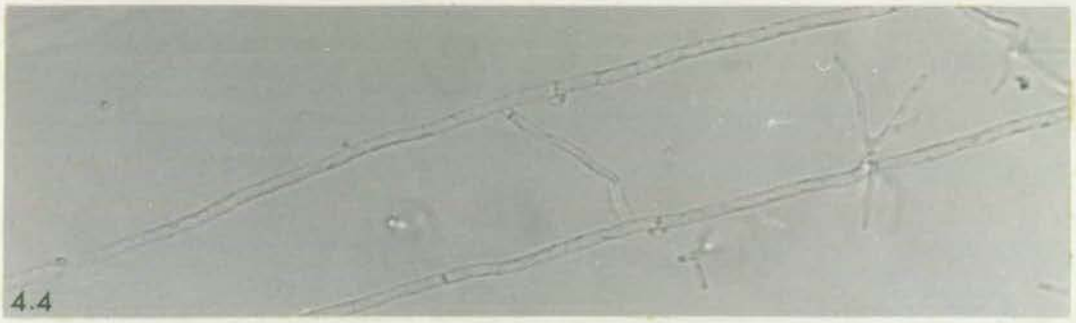
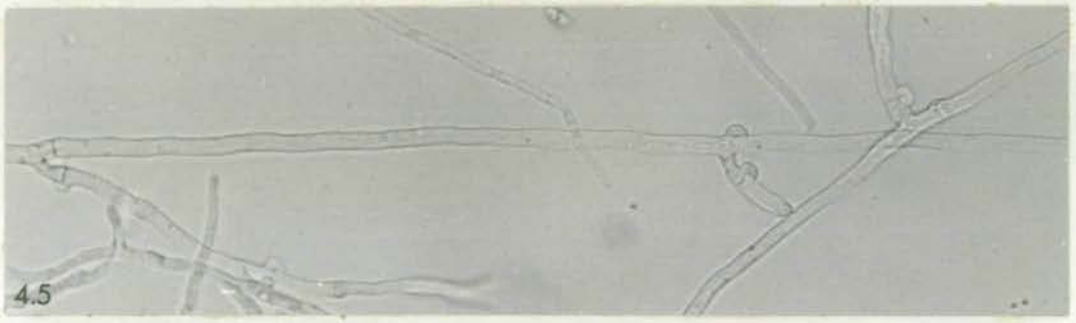


Fig. 4.2 Results of mating 15 monokaryons of Isolate 59 (P.'fimetaria' = P.coprophila Breeding Group II) in all combinations. (b = 'blocker' monokaryon; other symbols as in Table 4.3).

(a) Original mating table using Method I
(b) Rearranged mating table using Method I
(c) Original mating table using Method II
(d) Rearranged mating table using Method II





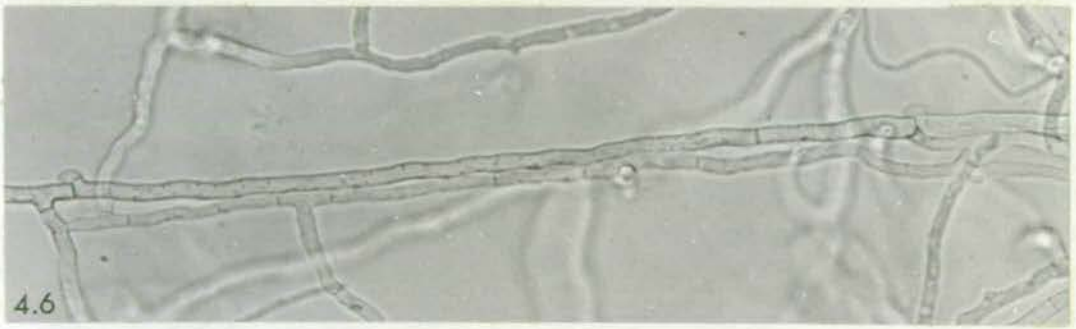


Fig. 4.3 Dikaryotic mycelium of Isolate 84 showing true clamps. (x 630)

Fig. 4.4 Common-B heterokaryotic mycelium of Isolate 84 showing pseudoclamps. (x 630)

Fig. 4.5 Dikaryotic mycelium of Isolate 59 showing true clamps. (x 630)

Fig. 4.6 Common-B heterokaryotic mycelium of Isolate 59 showing pseudoclamps. (x 630)

Fig. 4.7

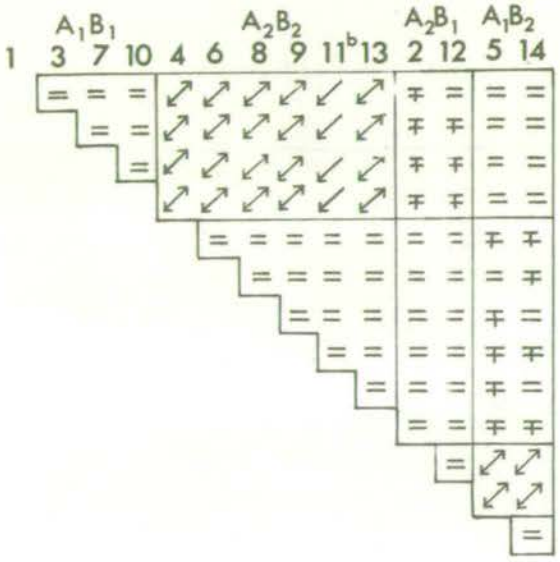
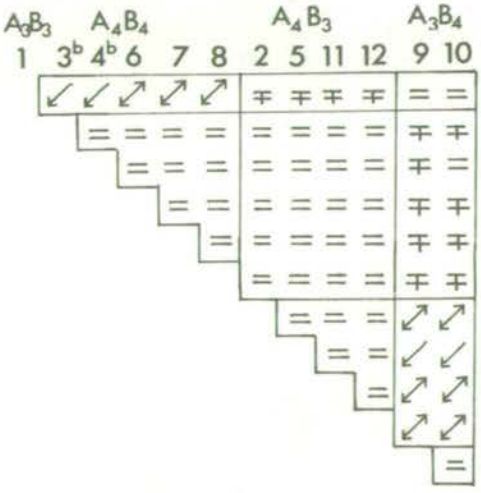


Fig. 4.8



Rearranged mating tables of isolates belonging to P.gracilis Breeding Group I. (b - 'blocker' monokaryon; other symbols as in Table 4.3).

Fig. 4.7 Mating table of Isolate 5.

Fig. 4.8 Mating table of Isolate 110.

Fig. 4.9

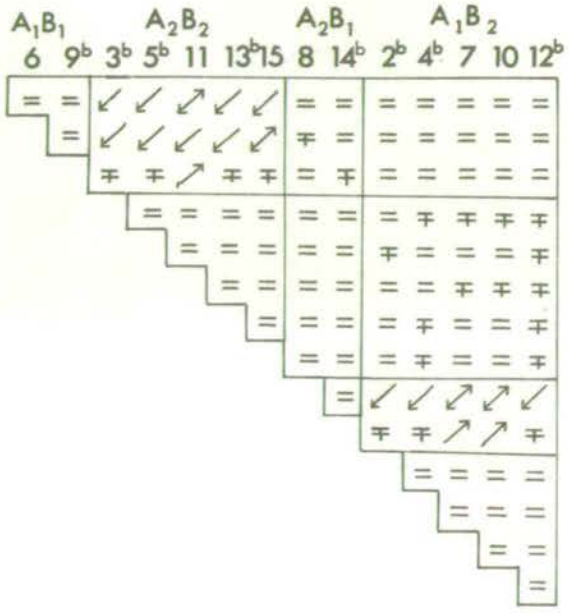
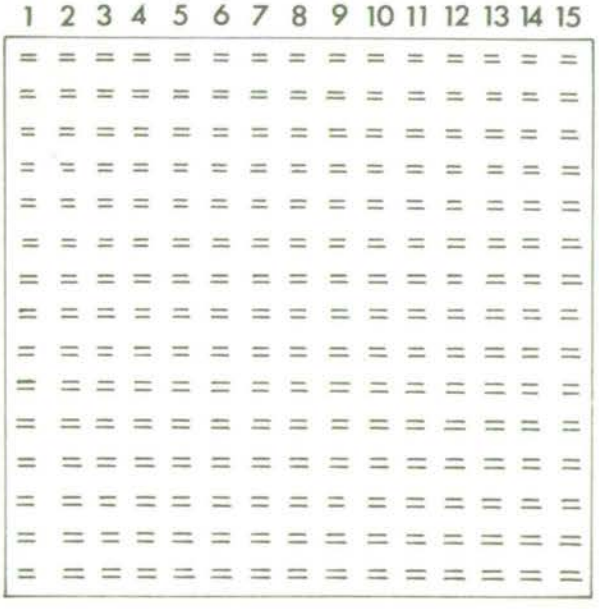


Fig. 4.10



Mating tables of isolates belonging to P.gracilis Breeding Group II. (b - 'blocker' monokaryon; other symbols as in Table 4.3).

Fig. 4.9 Normal rearranged mating table of Isolate 121.

Fig. 4.10 Mating table of Isolate 15 showing complete absence of compatible matings.

Fig. 4.11

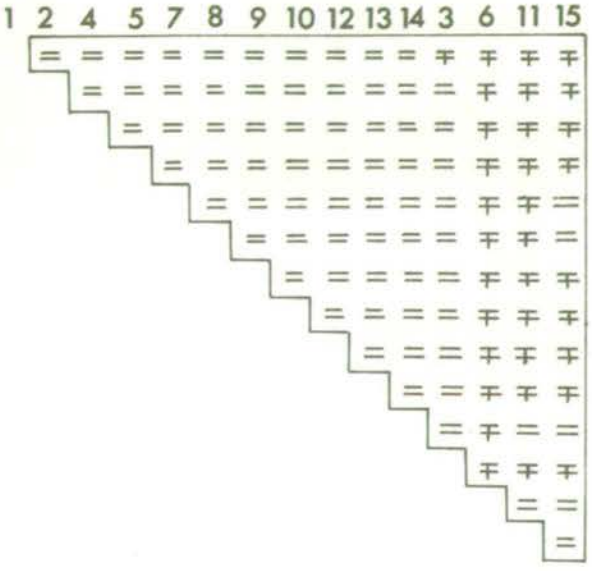
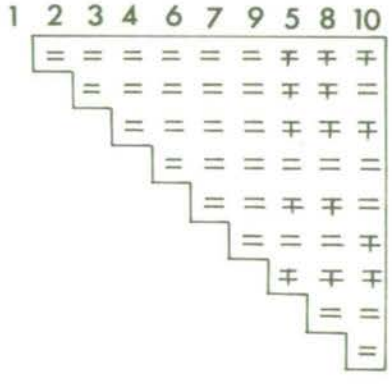


Fig. 4.12



Mating tables of isolates belonging to P.gracilis Breeding Group III showing complete absence of nuclear migration. (Symbols as in Table 4.3).

Fig. 4.11 Mating table of Isolate 101.

Fig. 4.12 Mating table of Isolate 104.

Fig. 4.13

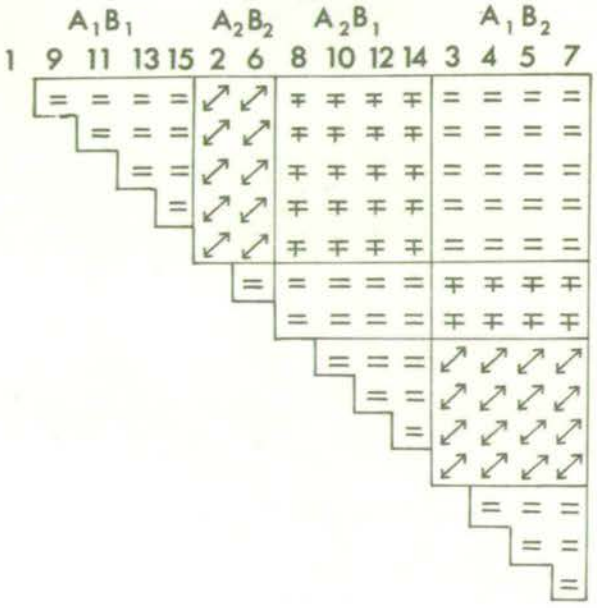
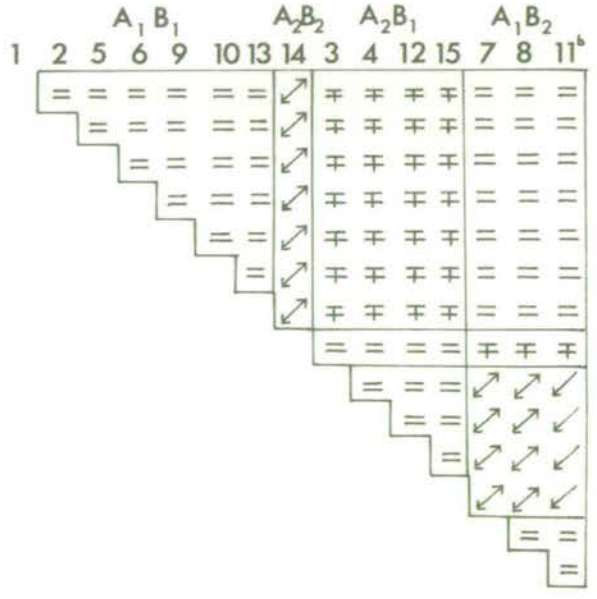


Fig. 4. 14



Rearranged mating tables of isolates belonging to the P.candolleana complex. (b - 'blocker' monokaryon; other symbols as in Table 4.3).

Fig. 4.13 Mating table of Isolate 141 (Breeding Group I).

Fig. 4.14 Mating table of Isolate 142 (Breeding Group II).

Fig. 4.15

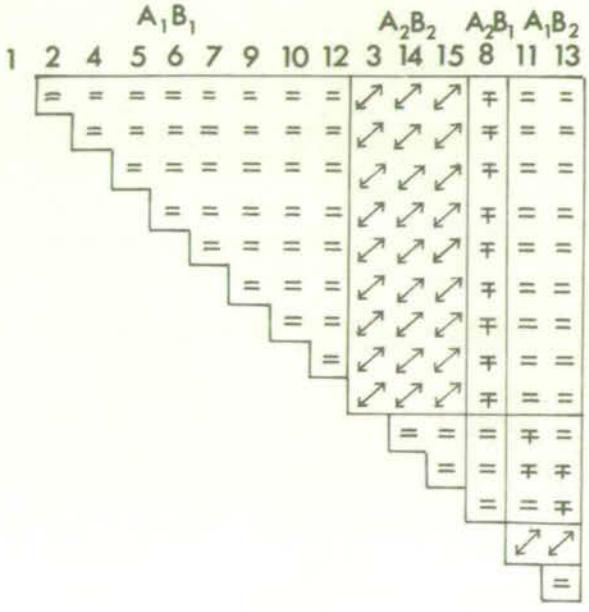
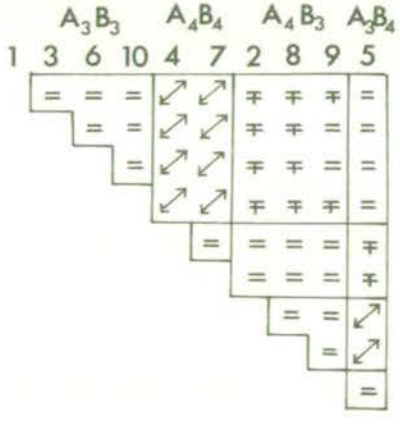


Fig. 4.16



Rearranged mating tables of isolates belonging to P.coprobia Breeding Group II. (Symbols as in Table 4.3).

Fig. 4.15 Mating table of Isolate 133.

Fig. 4.15 Mating table of Isolate 33.

The results of incompatibility systems of Psathyrella isolates are summarised in Table 4.4.

Table 4.4. Summary of incompatibility systems of several different Psathyrella isolates. (Symbols are explained in Table 4.3).

Breeding Group	Isolate Culture No.	Number of mating types	Migration patterns in compatible matings	Fig. No.
P.gracilis I	5	4	↗↙↗	4.7
P.gracilis I	110	4	↗↗↙	4.8
P.gracilis II P.microrrhiza	121	4	↗↗↙	4.9
P.gracilis II P.microrrhiza	15	1	none	4.10
P.gracilis III	101	-	none	4.11
P.gracilis III	104	-	none	4.12
P.candolleana I	141	4	↗	4.13
P.candolleana II	142	4	↗↗↙	4.14
P.coprophila I	84	4	↗	4.1
P.coprophila II "P.fimetaria"	59	4	↗↗↙	4.2
P.coprobia I	33	4	↗	4.16
P.coprobia I	133	4	↗	4.15
P.coprobia II	14	8	↗	5.1

The bifactorial results obtained for isolates of the P.gracilis complex agree with the results of Quintanilha, Quintanilha and Vasermanis (1941) and Quintanilha (1944). The bifactorial results obtained for isolates of the P.candolleana complex agree with the results of Vandendries and Brodie (1933); Quintanilha, Quintanilha and Vasermanis (1941) and Galland (1970).

Table 4.4 shows that out of thirteen isolates, only five had

typical bifactorial incompatibility system. In the remaining isolates several different modifications were found. Some of these are discussed in detail in the next section.

4.4 Modifications from the basic systems of incompatibility

4.4.1 Restricted nuclear migration

In five out of the thirteen isolates, in which incompatibility systems were analysed (Nos. 5, 110, 121, 142, 59), the basic pattern of bifactorial incompatibility was modified by the occurrence of matings with unilateral dikaryotisation. In some compatible matings one monokaryon of the pair always donated nuclei but was never dikaryotised in return. The monokaryons were consequently referred to as the 'blocker' strains. Morphologically they were indistinguishable from the wild type monokaryons.

When a wild type was mated with a blocker, unilateral migration was the result. When two blocker mycelia were mated, nuclear migration was absent, clamps were formed at the junction line only, and it was then impossible to distinguish the result from that of a common-B heterokaryon mating. The blocker mycelia were however clearly identifiable when a complete mating table was drawn up.

The behaviour of the blocker monokaryon Cult.No.142.11 was examined in matings with randomly chosen monokaryons of other compatible isolates (Table 4.5).

Table 4.5 Results of mating monokaryon 142.11 with other monokaryons of compatible isolates.

	136				146				171			
	1	2	3	4	1	2	3	4	1	2	3	4
142.11	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗

A unilateral migration pattern was obtained in each compatible mating

irrespective of whether the opposing mycelium was derived from the same or a different isolate.

The occurrence of unilateral dikaryotisation was first recognised by Brodie (1948) in some matings of Cyathus stercoreus. Brodie did not suggest why nuclear migration took place in one direction only.

Koltin (1970) described strains of Schizophyllum commune showing unilateral migration similar to that described in this study. He attributed the unusual mating behaviour to a 'modifier' mutation located outside the incompatibility factors. The 'modifier' and the 'blocker' mutations are clearly similar. Kemp (personal communication) found blocker mutations in Coprinus congregatus. His extensive investigations on complete tetrads of his bipolar species showed that the mutations were not linked to each other or to the single mating type locus.

It is interesting to consider why blocker mutants are common in natural populations of Psathyrella, and why natural selection maintains them. One possible advantage of restricted nuclear migration became apparent during observations on fruit-body production in culture (Section 10.4). If a number of monokaryotic mycelia of opposite mating type were inoculated together, fruit-body production was more successful than on agar plates with the same number of dikaryotic inocula. Similar observations were made by Kemp (personal communication) in Coprinus. They suggest that small patches of a composite mycelium consisting of dikaryotic and monokaryotic hyphae may provide conditions which are better suited for fruit-body production.

4.4.2 Absence of nuclear migration

In two isolates of P.gracilis Breeding Group III(Isolates 101

and 104) the incompatibility system could not be determined because nuclear migration never took place. Clamps were formed in some matings but were always confined to the junction line. When 4 monokaryons of Isolate 101 were crossed with 4 monokaryons of Isolate 104 nuclear migration failed in all 16 matings. As in other Psathyrella isolates, clamps and pseudo-clamps were not distinguishable and it was therefore not possible to assign mating types. The irregular mating behaviour can be explained if it is assumed that each monokaryon has a blocker allele.

In the previous section it was suggested that fruit-body production may be favoured by genetically imposed restrictions on nuclear migration. A fruit-body produced from a double blocker mating, e.g.Culture No.121.2 x 121.14 would be expected to give a mating table similar to that obtained in Isolates 101 and 104. It is unlikely to have been a coincidence that both the isolates of Breeding Group III which were sampled had a double dose of the mutant allele, especially as nuclear migration has never been observed in any of the matings between other isolates of this breeding group (Section 8.3). It seems more likely that the failure of nuclear migration is a regular feature of all the isolates of this breeding group and that the blocker mutants have some kind of selective advantage.

4.4.3 Absence of compatible matings

Isolate 15 was exceptional in that no compatible matings were obtained when 15 monokaryotic mycelia were mated in all combinations (Fig. 4.10). 225 matings were thus examined. In every mating samples from the area of contact were subcultured, and each grew to form a mycelium which was indistinguishable from the original monokaryons. The 15

original monokaryons were similar in their morphology. The possibility that only one mating type was sampled from spore progeny that is regularly bifactorial can be discounted since the statistical probability of this happening after sampling 15 monokaryons has been worked out to be less than 0.001% (Table 4.6).

Table 4.6 Percentage probability of finding only one mating type in samples drawn from a population consisting of mating types in four equal classes.

Sample size	1	2	3	4	5	6	7	8	9	10
% probability	100	25	6.250	1.563	0.391	0.098	0.024	0.006	0.002	0.001

Compatible matings were formed when monokaryons of Isolate 15 were mated with monokaryons obtained from other isolates of the same breeding group. When these isolates were mated within themselves the bifactorial pattern of incompatibility was always obtained.

The most likely explanation for the peculiar mating behaviour between monokaryons derived from Isolate 15 is that the original fruit-body was monokaryotic and so produced spores of one mating type. It is interesting that the spores of this isolate are larger than those of other isolates of the same breeding group (Section 8.4.2). Unfortunately it was impossible to prove the monokaryotic nature of the fruit-body since no attempt was made to propagate its tissues when fresh. The herbarium specimen was examined for the presence of clamps, and none was found. However this information is of no great significance since clamps were not always found on the herbarium specimens of normal dikaryotic fruit-bodies.

Lange (1952) has claimed that monokaryotic fruit-bodies of *Coprinus* have never been collected from the wild. The evidence given above shows that Isolate 15 may be an example of a monokaryotic *Psathyrella* which was found growing in its natural habitat.

CHAPTER 5 : A NEW SYSTEM OF INCOMPATIBILITY DETERMINED BY THREE FACTORS

5.1 Introduction

In an isolate with a unifactorial incompatibility system each monokaryon can mate with half of its sister monokaryons. In an isolate with a bifactorial incompatibility system each monokaryon can mate with a quarter of its sister monokaryons. The presence of a third factor would reduce sister mating even further to one in eight. This restriction in the level of inbreeding is presumably of evolutionary advantage and there is theoretically no reason why a trifactorial system should not exist. The results described below suggest that such a system may exist in an isolate of Psathyrella.

5.2 Materials and methods

The original isolate (No.14, P.coprobia, Breeding Group III) was collected by R.F.O. Kemp from horse dung found at Penicuik, Midlothian in April, 1967. The specimen was initially identified as Psathyrella coprobia by R. Watling and was placed in the herbarium of the Royal Botanic Garden, Edinburgh, under accession number R.W. 5243. No other isolates belonging to the same breeding group were found. In December 1969 a dikaryotic mycelium was obtained from a mass plating of basidiospores taken from the herbarium. The monokaryotic components of this mycelium were isolated by maceration in May 1970, and shortly afterwards fruit-bodies were formed in culture. These were harvested, dried and stored in silica gel. In November 1970 spores from one of these fruit-bodies were germinated to initiate the monokaryotic stocks which were used in this study. A total of 73 monokaryons were isolated and these were mated together in various combinations as described in Section 5.3.

Horse dung extract medium was used in all the experiments.

Mycelia were mated as described in Section 4.2.2 and scored as described in Section 4.2.3 (Method II).

In the growth rate experiment small plugs of mycelia were taken from the junction line of each mating, were inoculated 4 per plate, and were incubated for 4 days. The diameter of each colony was measured in mm.

In the experiment involving fruit-body formation plates were inoculated with 9 monokaryons in the form of a 3 x 3 square. The four corner inocula and the central one were of one mating type and the middle inoculum on each of the four sides was of the other type. The plates were incubated in the dark until the mycelia had met and were then transferred into the light at a room temperature of approximately 20°C. They were examined daily for the formation of primordia and mature fruit-bodies over a period of four weeks.

5.3 Results

5.3.1 Mycelial mating

After mating a random sample of 15 monokaryons in all combinations 7 different mating types were found and not four as expected in a bifactorial system. An eighth mating type was identified when representatives of the 7 known mating types were mated with a further sample of monokaryons. A total of 25 monokaryons, including representatives of the 8 mating types were then again mated in all combinations and the results are shown in Fig.5.1. The table has been arranged so that strains having the same mating type are next to each other. From the table it can be seen that:

1) There are 4 classes of matings in which clamp connections were isolated from both sides of the junction line. This indicated that nuclear migration took place.

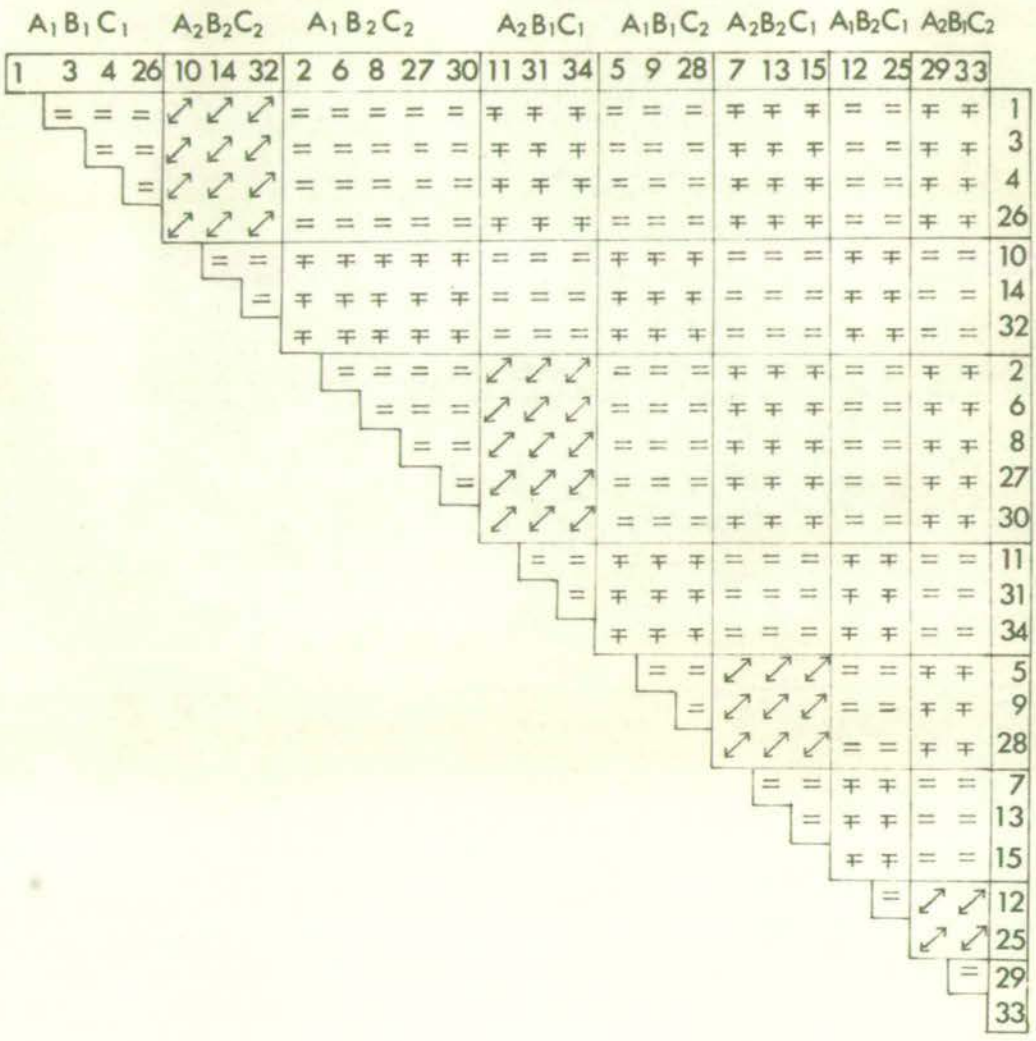


Fig. 5.1 Results of mating 25 monokaryotic mycelia of Isolate 14 (P.coprobia Breeding Group III) in all combinations.

↙↗ dikaryotic mycelia isolated from both sides of the junction line.

∓ heterokaryotic mycelia bearing false clamp connections isolated only from the junction lines

= matings indistinguishable from the monokaryons.

2) There are 12 classes of matings from which heterokaryotic hyphae bearing clamps were isolated only from the junction line. Detailed microscopic observations showed that the clamps were false.

3) There are 12 classes of matings which were indistinguishable from the monokaryons.

It is impossible to explain these results by a system of incompatibility based on only two factors. The possibility that the original fruit-body was contaminated with spores of neighbouring fruit-bodies has been considered, but then different results from those obtained here would be expected.

For a single fruit-body to produce monokaryons of two mating types, only one factor is necessary for their determination. For a single fruit-body to produce monokaryons of four mating types, two factors are necessary for their determination. In a system with monokaryons of eight mating type classes three factors are probably involved. A trifactorial system has never before been found in the fungi. As the two factors of a bifactorial system have been given the symbols A and B the additional factor present in a trifactorial system has been designated C. Further studies may reveal that the two systems are not homologous. Mating types have been assigned to the monokaryons on this basis and the eight combinations of mating type alleles are shown along the top of Fig.5.1.

In order to make possible a more detailed analysis of the results, it is necessary to reconsider some of the characteristics of bifactorial incompatibility revealed by the studies of Raper (1968) in Schizophyllum

commune. Heterozygosity for the A factor (A≠B=) results in the formation of heterokaryotic mycelia with pseudoclamps at the junction line. No nuclear migration takes place and the hyphae which bear pseudoclamps often form a ridge at the junction line which is known as a 'barrage'. When the mated mycelia are heterozygous for the B factor (A=B≠) nuclear migration can occur into both of the established monokaryotic mycelia but the resulting heterokaryotic mycelium has no clamps or pseudoclamps. In Schizophyllum these mycelia can be distinguished by their flat morphology (Papazian, 1950).

A comparison between the mating characteristics in bifactorial and trifactorial isolates is summarised in Table 5.1.

Table 5.1 The genotypes and mating characteristics of bifactorial and trifactorial systems of incompatibility. The symbols used are those of Raper (1966).

Bifactorial		A≠B≠ Compatible	A≠B= False clamps "Barrage"	A=B≠ Migration "Flat"	A=B=
Trifactorial	C≠	A≠B≠C≠ Compatible	A≠B=C≠ False clamps	A=B≠C≠= As mono-karyon	A=B=C≠ As monokaryon
	C=	A≠B≠C= False clamps	A≠B=C= False clamps	A=B≠C= As mono-karyon	A=B=C

Table 5.1 shows that in a bifactorial system there are four possible combinations of factors and that there are at least three different mating reactions. The functions of the A and B factors can therefore be distinguished. In the trifactorial system there are eight combinations of factors, but only three different mating reactions. The two systems are similar in that mycelia bearing pseudoclamps can be isolated from the

junction line in the matings which involve different A factors. However, whereas in the bifactorial system only two factors have to be heterozygous for the formation of heterokaryotic mycelia throughout both the initial mycelia, all three factors have to be heterozygous in a trifactorial system. In the bifactorial system, heterozygosity for the B factor alone is sufficient to permit nuclear migration. In Schizophyllum heterokaryons of the genotype A=B≠ can be distinguished by their flat morphology. In common-A matings of trifactorial systems where either or both of the B and C matings were heterozygous, mycelia with a characteristic growth form were not isolated.

From the above information it can be concluded that in the trifactorial system, as in the bifactorial system, the A factor is largely responsible for the formation of clamp connections. More information is however needed to distinguish between the functions of the other two factors. Further experiments were therefore carried out.

5.3.2 Nuclear migration experiment

In order to determine whether nuclear migration takes place in the common-A-matings of the trifactorial system, eight different common-A matings were set up. After the mycelial matings had been in contact for several days sample plugs from the peripheries of the colonies were isolated and used in further mating tests. The sample plugs were each paired with two mating types with genotypes opposite to that of the original mating. For example, if the original mating was A1 B1 C1 x A1 B2 C2 then the plugs from the peripheries were each mated with A2 B2 C2 and A2 B1 C1. Compatible matings resulted only if the peripheral sample plug was mated with a mating type opposite to that of the original colony. For example

a sample plug from the periphery of the original A1 B1 C1 colony gave a compatible result only if mated with A2 B2 C2. This indicated that nuclear migration did not take place between the original mycelia of the common-A mating. It seems that all three factors must be different for nuclear migration to occur.

During the analysis of mating types it was noticed that sample plugs taken from the various matings had a number of characteristic growth rates. Mycelial growth rate and fruiting experiments were therefore carried out to test this observation in an attempt to obtain more information about the functions of the B and C factors.

5.3.3 Mycelial growth rate and heterokaryon stability

Two monokaryons of each mating type were selected and mated in all combinations. Small plugs of mycelia were taken from the junction line of each mating and were inoculated, incubated and measured as described in Section 5.2. The diameter of the mycelia after 4 days' growth are shown in Table 5.2. The table shows that the samples derived from the matings which resulted in the formation of clamp connections were of two sizes. The dikaryons (A≠B≠C≠) and the heterokaryons (A≠B≠C=) had a mean diameter of 32.56 and 32.00 mm respectively. The heterokaryons of genotypes A≠B=C≠ and A≠B=C= had a mean diameter of 23.00 and 21.25 mm respectively. The difference between the diameters of the 'fast' and 'slow' clamp bearing mycelia was shown to be very highly significant by an analysis of variance. The growth rates of the A= heterokaryons were rather variable and were not analysed.

Mycelia were isolated from the junction line of all 120 matings made in the growth rate experiment, and were stored in bijoux bottles.

Table 5.2 The relative growth rates (mm) of mycelia isolated from the junction-line of a mating between two members of each of the 8 mating types.

A1B1C1		A2B2C2		A1B2C2		A2B1C1		A2B1C2		A2B2C1		A1B2C1		A2B1C2		
3	4	10	14	6	8	11	34	5	9	7	13	12	25	29	33	
	26	34	32	26	29	23	26	30	30	30	31	27	28	23	23	3
		30	31	28	30	19	22	31	33	30	30	25	25	21	19	4
			28	19	21	25	26	34	35	34	35	25	24	29	30	10
				19	22	30	30	31	32	30	27	21	25	30	10	14
					25	30	33	26	27	24	20	25	26	33	35	6
						31	34	33	33	26	24	31	32	31	32	8
							28	23	22	31	30	31	32	30	33	11
								25	23	31	29	35	34	29	29	34
									30	33	33	28	26	23	21	5
										34	34	28	28	22	22	9
											30	19	21	27	28	7
												18	23	27	28	13
													30	32	33	12
														31	33	25
															29	29
																33

fast growing matings in which nuclear migration, true clamp formation and mature fruit-body production takes place.

fast growing matings in which pseudoclamps are formed at the junction line.

slow growing matings in which pseudoclamps are formed at the junction line.

When the mycelia was subcultured after 2 or 3 weeks of storage it was found that in some matings pseudoclamps were not recovered. Pseudoclamps were recovered in all A≠B≠C= matings but not in matings of genotype A≠B=C= and A≠B=C≠. On the basis of these results it is possible to conclude that while pseudoclamps are formed in all different-A heterokaryons, the stability of the pseudoclamps depends on both the A and B factors. Stable pseudoclamps are only formed when both the factors are different.

5.3.4 Fruit-body production

In order to test whether heterozygosity for all three factors is necessary for the formation of fruit-bodies, plates were inoculated with monokaryotic inocula as described in Section 5.2. The inocula were inoculated according to this pattern because it has been found (Section 10.4) that fruiting occurs more readily when two monokaryotic inocula come in contact than when the plate is inoculated with one or more dikaryotic inocula only.

Mature fruit-bodies were formed in five pairs of matings all of which were heterozygous for the three mating type factors. A photograph of a fruit-body is included in Chapter 10 (Fig.10.12). The matings of the type A≠B≠C= all developed small primordia but no mature fruit-bodies were formed. All other matings failed to produce any primordia. By referring to Table 5.2 it can be seen that the fast-growing heterokaryons with pseudoclamps, which were isolated from the junction line, were those which produced primordia and these all had the genotype A≠B=C=. The slow-growing heterokaryons with pseudoclamps which were A≠B=C= or A≠B=C≠ developed no primordia. It is therefore possible to conclude that

primordium initiation only takes place in matings in which the B factor is different. The heterokaryons with the genotype A≠B≠C= although growing at the same rate as the fully compatible matings are distinguishable morphologically by their side branches which often grow in contact with the main axis instead of at an angle of about 45°. This gives the periphery of the colony a rather spiky appearance.

5.3.5 The relative position of the three factors on the chromosomes

In a bifactorial isolate sister mating occurs at the level of one in four if the intra-factor recombination is low. All bifactorial isolates show independent segregation of the A and B factors. The reduction in the level of sister mating is thought to be the evolutionary advantage of the bifactorial system over a unifactorial one. The halving of sister mating with each additional locus will only occur if the factors segregate independently.

73 monokaryons were analysed for mating type and therefore an indication of the linkage relationships of the three factors is possible. The total number of monokaryons in the eight mating type classes is shown on Table 5.3. If the three factors are not linked the number of monokaryons in each of the mating type classes should be equal. A chi-square test was therefore carried out to test whether the observed totals of each class differed significantly from the mean of 9.125. The result showed that there was no significant difference from the mean (χ^2 = 6.67 with 7 D.F; P = 0.5-0.3). The result for this small sample suggests that the three factors are inherited independently.

Table 5.3 The total numbers of monokaryons in the eight mating type classes.

Mating type			Total
A1	B1	C1	8
A2	B2	C2	8
A1	B2	C2	15
A2	B1	C1	8
A1	B1	C2	9
A2	B2	C1	12
A1	B2	C1	6
A2	B1	C2	7
			73

5.4 Discussion

Using all the available information it is possible to distinguish 4 different reactions from among the 8 heterokaryon classes. The reactions are summarised below.

1) A≠ B≠ C≠ stable true clamps; nuclear migration; fast growth rate; mature fruit-bodies.

2) A≠ B≠ C= stable pseudoclamps; no nuclear migration; fast growth rate; fruit-body primordia.

3) A≠ B= C= unstable pseudoclamps; no nuclear migration; slow growth rate; no fruit bodies or primordia.
A≠ B= C≠

4) A= B≠ C≠
A= B≠ C= no pseudoclamps or true clamps; no nuclear migration; variable growth rate; no fruit-bodies or primordia.
A= B= C≠
A= B= C=

The following conclusions can thus be made. The A factor is con-

cerned with most aspects of clamp formation and is similar in function to the A factor of a bifactorial system. The B factor is concerned with the initiation of primordia while all three factors must be different for nuclear migration and for the formation of mature fruit-bodies. However, it is still not possible to detect an independent function for C. It is possible that the function of C is epistatic to both the A and B functions, so that the effect of the C factor would only be detected in compatible matings.

After the publication of these findings (Jurand and Kemp, 1973), Raper (unpublished) criticised the interpretation that three factors are involved. He suggested that the usual pattern of bifactorial incompatibility is here modified by a mutation or mutations located outside the two incompatibility factors. Many modifier mutations have been described by Raper and his coworkers in cultured strains of Schizophyllum commune, but none were collected from naturally growing fruit-bodies and none gave a pattern of mating which is as clear and unambiguous as that presented here. Raper and Raper (1964) found that the normal bifactorial mating pattern can be altered by the presence of an A factor modifier gene which converts the common-A heterokaryons into different-A mimics. Raper suggested that a similar 'aberration' could be responsible for the present results. However, neither a dominant or recessive A or B factor modifier could possibly explain the results shown on Fig.5.1. The impressive feature of this table is the regular 1:1 distribution of clampy (or pseudoclampy) heterokaryons to those bearing no clamps (or pseudoclamps). If A or B factor modifiers were present an unequal distribution would be expected.

The modifier genes studied by Koltin (1970) disrupt nuclear migration so that strains carrying the mutation donate nuclei in mating interactions but never accept nuclei in return. These modifiers are clearly similar to the blocker mutants described in Section 4.4.1. Blocker mutations cannot be responsible for the results given in this chapter since none of the matings showed the unilateral migration pattern.

The system of incompatibility here described is undoubtedly determined at three loci. The controversial question is whether the third locus is an independent incompatibility factor or whether it is simply an independent locus bearing a chance mutation which modifies the incompatibility system. Because it is not possible to differentiate between common-C1 and common-C2 heterokaryons it is unlikely that a chance mutation is involved. If a mutant were involved one would expect to be able to differentiate between a double dose of the mutant allele and a double dose of the wild-type. An analysis of the F_2 generation might provide further evidence, but much work would be involved since the progeny of all four fully compatible matings would have to be analysed. It is unfortunate that other isolates of the same breeding group are not available.

CHAPTER 6 : LIGHT AND ELECTRON MICROSCOPY INVESTIGATIONS ON THE STRUCTURE OF OIDIA

6.1 Introduction

The existence of oidia has long been known (Falck, 1902; Brodie, 1936; Chow, 1934). They are formed mitotically, are uninucleate and are usually borne on monokaryotic mycelia of heterothallic basidiomycetes. Oidia of most species are hyaline, 2-10 µm long, and are formed in heads or clusters on oidiophores. In some species the clusters cohere in a droplet of mucilage and the oidia are then known as 'wet oidia'. Other species have 'dry oidia' which remain distinct in Penicillium-like chains. Chow (1934) described several different methods of oidium development in the genus Coprinus and noted that their method of formation and their size is constant for each species.

Oidia of different species may have different functions. In some species they germinate readily to form monokaryotic mycelia. In many species, particularly coprophilous ones (Falck, 1902) they do not germinate but act as fertilizing agents or spermatia. Recently Kemp (1970) and shortly afterwards Bistis (1970) briefly drew attention to an interesting reaction between hyphae and oidia. When an oidium is placed in the vicinity of a hypha the tip responds to an oidial stimulus and directed growth followed by fusion takes place. The physiological basis of the recognition of oidia by hyphae has not yet been resolved and a study of oidial structure in isolates of the genus Psathyrella may be relevant to this problem.

6.2 Production of oidia in culture

Oidia were formed on monokaryotic mycelia of most isolates when plates were kept at room temperature. In no isolates were oidia produced

on dikaryotic mycelia. In a few isolates oidia were never formed. The ability or failure of an isolate to produce oidia in culture could not be used as a taxonomic character since it was found to depend on both the method of culture and the genotype.

In most isolates oidia were formed abundantly on young monokaryotic mycelia grown from freshly germinated basidiospores. In most coprophilous isolates oidial production remained abundant throughout the life of the stocks. In soil-growing isolates particularly those belonging to the P.candolleana and P.gracilis complexes the ability of monokaryons to form oidia was lost with repeated subculturing. In some of these stocks it was found that the ability to form oidia could be regained by storing the mycelium at a low temperature. Abundant oidia were formed when monokaryotic cultures were transferred from room temperature to a cold room at $4^{\circ}C$ for two weeks. This observation is in agreement with the findings of Chow (1934) who suggested that conditions which are unsuitable for good vegetative growth often promote oidial production.

6.3 Light microscope observations on the development and structure of oidia

6.3.1 Method

Details of oidium development and oidophore morphology were observed directly on agar plates. Observations of oidium size and shape were made after relief staining according to the method of Duguid (1951). A drop of concentrated Indian ink was placed on a clean slide and a drop of oidial suspension added. The oidia were usually transferred from the culture plate in a droplet of sterile water held in a tungsten loop. Sometimes in order to obtain a higher concentration of oidia on the slide the oidia were concentrated by centrifugation (3000 rev./min. for 5 min.)

A large coverslip was gently lowered onto the slide and the oidia were examined using the oil immersion objective. At least 12 oidia of each isolate were drawn using a Camera Lucida.

6.3.2 Oidium development

In all Psathyrella isolates oidia were formed in 'wet' heads and the method of development was similar. Developing clusters of oidia of four different breeding groups are shown in Figs. 6.2-6.5. In these and other Psathyrella isolates oidia were formed by the septation of the finger-like outgrowths of the oidiophore (Fig.6.1). The oidiophore is the hypha on which oidial clusters develop, and the finger-like outgrowths are budded off by sequential branching. Each outgrowth then septates to give two or three oidia. The oidiophores, outgrowths, and the oidia are approximately of the same diameter as the vegetative hyphae. The sequence in the formation of septa was not studied in detail, but since only two oidia are most commonly formed per outgrowth such terms as 'basipetal' and 'acropetal' are hardly relevant. Oidia are formed by septation and are therefore a type of arthroconidia in the sense of Hughes (1953).

Galland (1973) also described oidiophore morphology in a number of Psathyrella species and her observations are similar to those of this study.

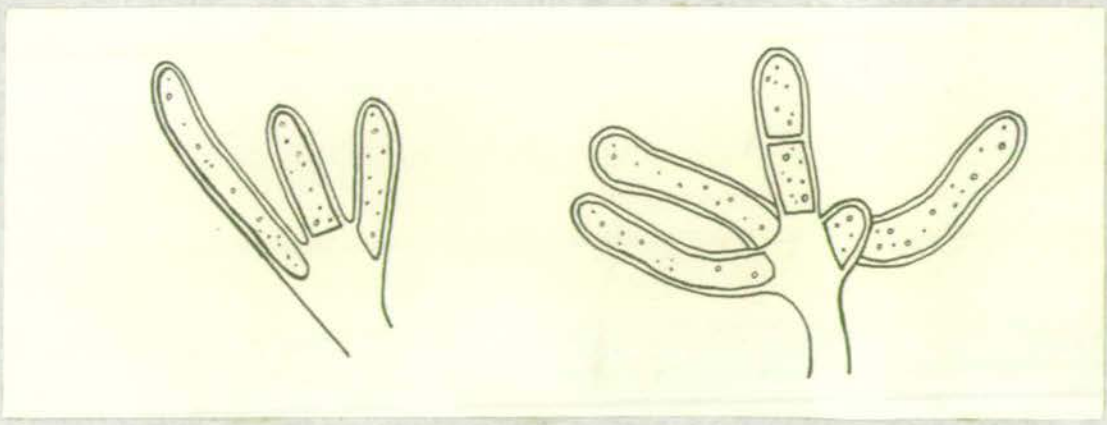


Fig. 6.1 Developing clusters of oidia in P.vernalis (Isolate 18) mounted in Indian ink and copied using a Camera lucida.

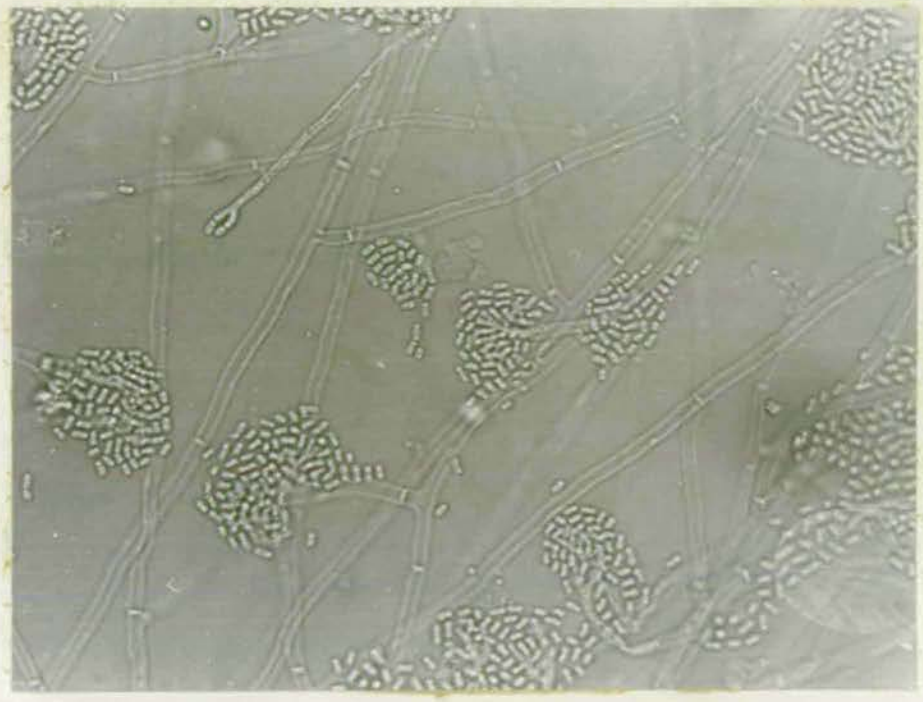


Fig. 6.2 Clusters of oidia of P.coprophila (Isolate 1). (x 830)

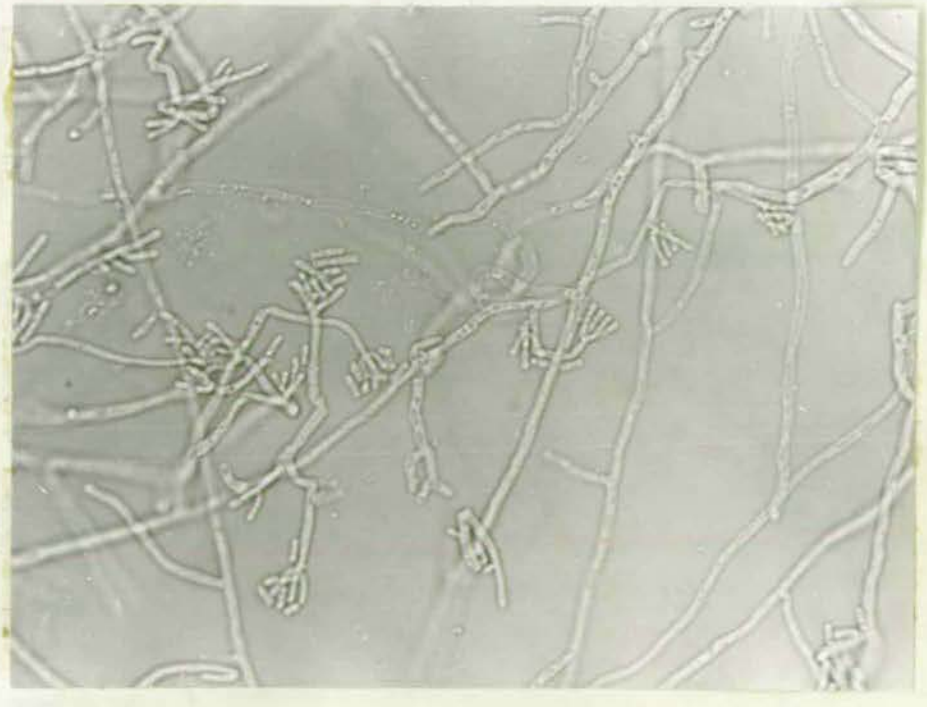


Fig. 6.3 Clusters of oidia of P.candolleana (Isolate 142). (x 830)

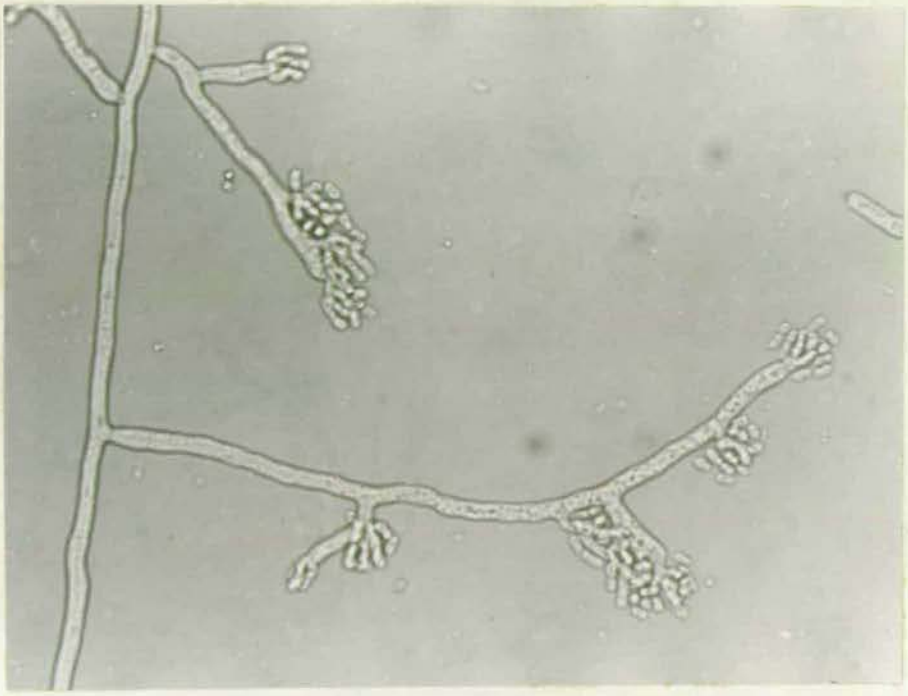


Fig. 6.4 Clusters of oidia of P.coprobia (Isolate 30) (x 830)

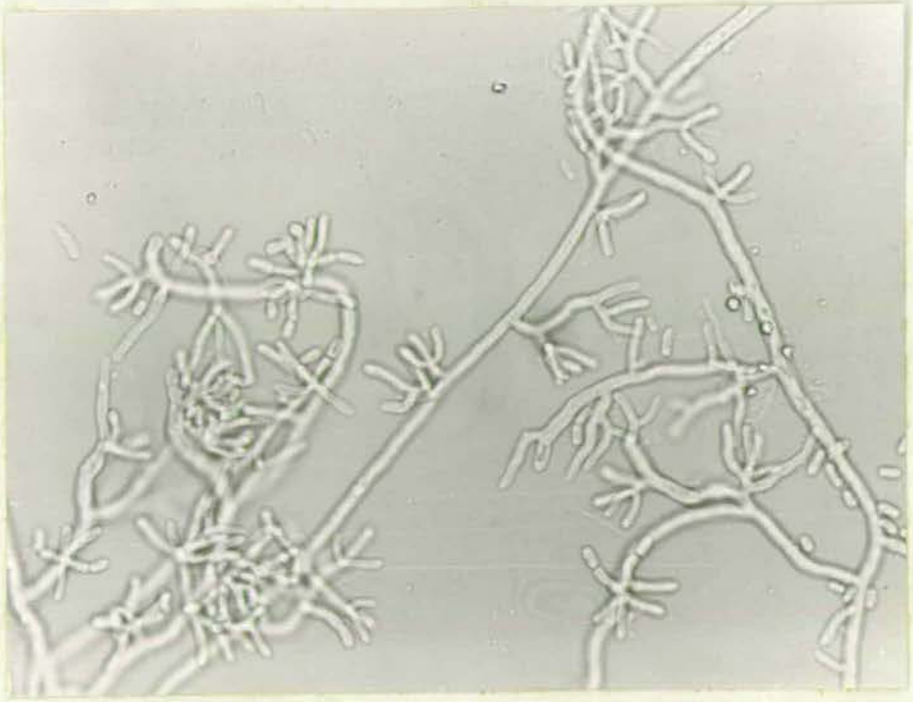


Fig. 6.5 Clusters of oidia of P.gracilis (Isolate 101) (x 830)

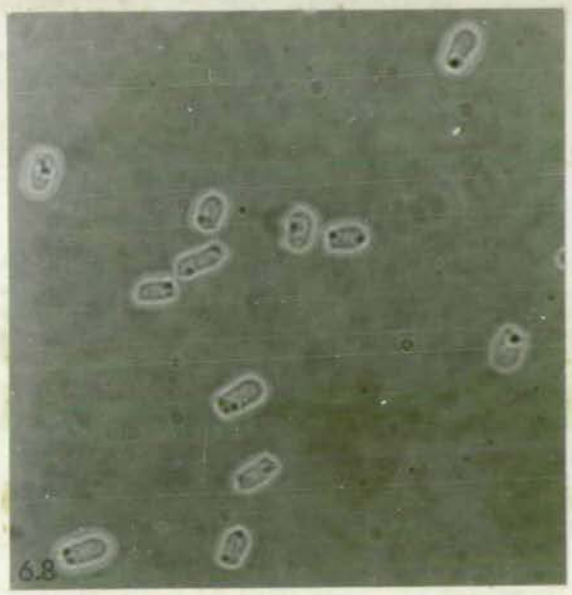
6.3.3 Size, shape and germination of oidia

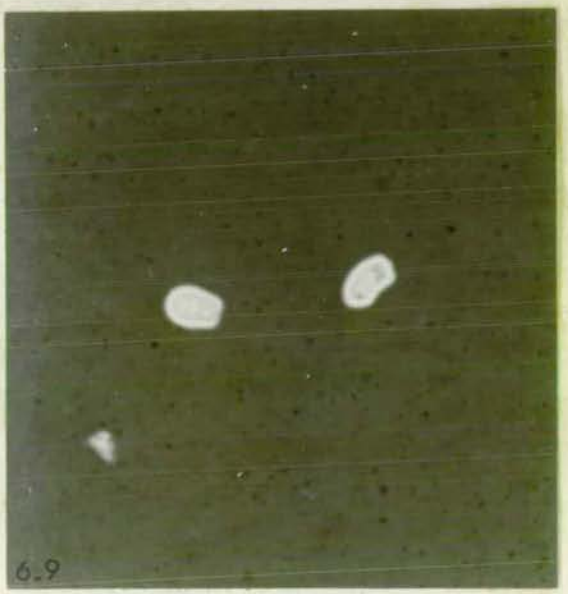
Light microscope photographs of oidia of four isolates belonging to different breeding groups are shown in Figs. 6.6-6.9. Germinating oidia of P.candolleana are shown on Fig.7.2. The average length and width measurements of oidia are given in Table 6.1. At least 12 oidia were measured in each sample. The ability of oidia to germinate on agar within a week of inoculation is noted in the same table.

Table 6.1 Average measurements and the germination ability of oidia of several different isolates (+).

Identification	Breeding Group	Isolate No.	Length (µm) Mean (Range)	Width (µm) Mean (Range)	Germination of oidia
P.gracilis	II	15	5.6(4.1- 8.9)	2.6(2.4-2.8)	not studied
"	"	17	6.8(4.7- 8.3)	2.7(2.4-3.3)	+
"	"	71	5.3(4.7- 6.1)	3.1(2.4-3.3)	not studied
"	"	151	6.0(4.1- 8.7)	2.7(2.0-3.0)	+
"	III	101	6.2(4.9- 9.5)	2.7(2.4-3.0)	+
"	"	105	5.4(4.9- 6.1)	2.6(2.2-3.0)	+
P.vernalis		18	8.2(5.3-13.0)	3.4(2.8-3.7)	+
P.candolleana	I	145	6.0(4.1-10.2)	2.2(2.0-2.4)	+
"	II	142	5.6(2.4- 9.6)	2.0(1.8-2.4)	+
P.coprophila	I	51	3.7(2.4- 4.1)	2.1(1.8-2.4)	nil
"	II	59	4.7(3.7- 6.5)	2.0(1.8-2.4)	nil
P.coprobia	II	133	4.0(3.0- 4.9)	2.5(2.0-3.0)	nil
P.spadiceo-grisea		8	4.4(3.3- 5.3)	3.1(2.6-3.7)	not studied

Table 6.1 shows that within each isolate oidial length is a variable character. Oidial width is more constant, since it depends on the width of the vegetative hypha. In most isolates the oidia were much longer than wide. Galland (1973) observed a more even length to width ratio in the oidia of P.spadiceo-grisea and this is also true of the





Photomicrographs of oidia relief stained in Indian ink. (x 2000)

Fig. 6.6 P.pygmaea (Isolate 94)
Fig. 6.7 P.candolleana (Isolate 142)
Fig. 6.8 P.coprophila (Isolate 1)
Fig. 6.9 P.coprobia (Isolate 30)

P.spadiceo-grisea isolate examined in this study.

It is interesting, but not surprising that the oidia which could germinate were on average larger than those which could not. Oidia of the coprophilous isolates always failed to germinate. In the non-coprophilous isolates some oidia did germinate on agar, but never more than 10-15%. This suggests that germination is not a primary function of oidia in this genus.

Figs. 6.6-6.9 show that a clear zone surrounds each oidium. A similar surface covering was found on the oidia of all the isolates examined. Its thickness is included in the measurements given in Table 6.1. The coat may be made of mucilage and may cause the oidia to cohere in 'wet' clusters. Oidia formed in 'dry' clusters e.g. Flammulina velutipes lack such a covering. Duguid (1951) described a similar mucilagenous layer on the surface of many bacteria and referred to it as 'the capsule'. He also found that many 'capsulate' bacteria have hair-like appendages called 'fimbriae' (Duguid, Smith, Dempster and Edmunds, 1955). Fimbriae are only visible in the electron microscope. Electron microscope investigations on oidia are described in the next section.

6.4 Electron microscope observations on the structure of oidia of P.coprophila

These findings are described briefly in the short communication (Jurand and Kemp, 1972). P.coprophila was chosen because it produced abundant oidia in culture. The type specimen (Isolate 1) was used.

6.4.1 Preparations for electron microscopy

Monokaryotic mycelia were grown on agar plates at 20°C on

horse dung extract agar. The oidia were harvested when the mycelium almost covered the agar surface. 0.81% (w/v) NaCl was used to harvest the oidia. About 4 ml of the saline solution was poured onto each mycelium and the oidia suspended by gentle rocking of the plate. The suspension was then centrifuged at 3000 rev./min for 5 min. to concentrate the oidia into a pellet. Three fixation methods were used to prepare oidia for sectioning. Negative staining and metal shadowing techniques were also used for the observation of whole, unsectioned oidia. The five methods of preparation are described in detail below.

(i) Fixation with OsO_4 (1%,w/v). The method used was according to Palade (1952) but with the addition of 0.045 mg sucrose/ml of fixative for 1 h at room temperature.

(ii) Double fixation with glutaraldehyde and OsO_4. The oidia were fixed for 1 h at 0 to 2°C with glutaraldehyde (2.5%, w/v) in 0.1 M-phosphate buffer at pH 7.2, then washed with 0.1 M-phosphate buffer and fixed with OsO_4 (1%,w/v) dissolved in the buffer.

(iii) Double fixation in the presence of ruthenium red. The fixation procedure used was that of Luft (1965). It has been described in detail by Pate and Ordal (1967).

After fixation methods (ii) and (iii) the pellets were rinsed with buffer, dehydrated with a graded series of alcohol solutions, passed through 1,2- epoxy propane and embedded in Araldite. Fixation method (i) was followed without rinsing by dehydration and embedding. Sections were prepared with glass knives on an L.K.B. Ultratone. Sections of the osmium fixed material were stained with 2.5% (w/v) uranyl acetate and 1% (w/v) potassium permanganate for 20 min. (Lawn, 1960). The sections of the double fixed material, methods (ii) and (iii), were stained with

1% (w/v) uranyl acetate solution for 30 min. and with lead citrate solution (Reynolds, 1963) for 15 min.

(iv) Negative staining with phosphotungstic acid. Oidia were transferred from the plate to the grid using a platinum loop immersed in 0.81% (w/v) NaCl. They were fixed while on the loop with osmic acid vapour. The loop was inserted into the mouth of a bottle containing 2% osmic acid for about 30 seconds. The oidia were then negatively stained on the grid with a drop of 4% (w/v) phosphotungstic acid at pH 7. The excess liquid was drawn off with filter paper and the grids inserted into the electron microscope.

(v) Shadow-casting with platinum-palladium. The method of Pate and Ordal (1967) was adopted as follows. 3.7% formaldehyde was used to harvest and fix the oidia. The cells were left in the fixative in ice for three hours and then concentrated by centrifugation. They were then suspended and centrifuged in three changes of 0.067 M-phosphate buffer in order to wash away the fixative, and the final pellet was resuspended to give a slightly turbid solution. Drops of the suspension were placed on electron microscope grids and the excess liquid was drawn off with filter paper. The grids were placed in a vacuum evaporator and a platinum-palladium wire was evaporated over them. Some of the grids were rotated to give an even, all round shadowing and others were shadowed at an angle of 26.5°. The grids were then examined in the electron microscope.

An AEI EM6B electron microscope was used to examine both the sectioned and whole material.

6.4.2 Results

Sections of oidia prepared after fixation with OsO_4 (method i)

and those prepared after double fixation (method ii) showed filamentous appendages on the surface of the oidia (Figs. 6.10-and 6.11). The filaments were similar in size and shape after both these methods of fixation. They were approximately 0.5 µm long with a diameter of 10 nm, and they appeared to taper slightly. The structure of the appendages differed after these two methods of fixation. After osmic fixation the filaments appeared to be hollow while those after double fixation appeared to be more solid and heavily stained.

Sections of oidia double fixed in the presence of ruthenium red (method iii) indicated the presence of a capsule similar to that seen in the light microscope preparations (Fig. 6.14). A fibrillar component was present in the ruthenium red positive region but the appendages were not distinct. The capsule probably contained some highly polymerised acid mucopolysaccharides because the affinity of ruthenium red to these surface carbohydrates is well documented (Luft, 1964). The ruthenium red positive region was similar in thickness to the light band which was seen in the light microscope preparations and to the length of the filaments seen after fixation methods (i) and (ii).

In some of the negatively stained preparations of whole, unsectioned oidia, (method iv), flexuous filamentous appendages were also found (Figs. 6.15 and 6.17). These filaments are however not nearly as distinctive as those seen in the sectioned material. Since they were found only in one or two preparations it is possible that the filaments seen in the negatively stained preparations are artifacts. They are, however, similar in diameter to the filaments found after methods of fixation i, ii and iii (Figs. 6.12-6.15).

There is no evidence of filamentous appendages in the metal shadowed preparations of whole, unsectioned oidia (Fig. 6.16). The strands which are seen to surround the oidia are branched in some preparations and are probably formed at random by the drying of mucilage. Any filamentous appendages that might have been present would be obscured by the formation of these strands. Similar strands were obtained in metal shadowed preparations of other fimbriate organisms. Tweedy, Park and Hodgkiss (1968) made similar cautious conclusions in some Vibrio spp. However, Yanagava and Otsuki (1970) in Corynebacterium renale and Wistreich and Baker (1971) in Neisseria spp consider the strands to represent true fimbriae.

The internal structure of oidia could be observed in the sectioned material, although only the major organelles were clearly distinguishable (Fig. 6.18). A single, large nucleus occupied almost half the cell sections. All the cell membranes and especially the nuclear envelope were most clearly shown after osmic fixation. The ribosomes, free in the cytoplasm, were especially clear in the double fixed sections. The mitochondria were large in proportion to the size of the oidium and were elongated and few in number (Fig.6.18). All the oidia contained at least one prominent inclusion which was also visible in the light microscope. These inclusions are probably lipid structures of the type referred to by Hawker (1965).

The shape of the oidia is more clearly shown than in the light microscope preparations. Some oidia have one flat end and one rounded end, while others have two flat ends. Oidia of the first type are produced as terminal sections of the finger-like outgrowths of the

oidiophore and the others are intercalary (Fig.6.19). The sides of the oidia and the rounded ends are bound by two cell walls, the original hyphal wall and the inner oidial wall. It is not clear whether the septal ends are bound by one wall only (Fig.6.10) or two walls (Fig.6.11). The hyphal cell walls usually project slightly to give the appearance of terminal flanges. The filamentous appendages are present all over the oidial surface, although they do appear to be less frequent on the flat septal ends.

6.4.3 Discussion

In this study several different techniques were tried in order to reveal the surface structure of oidia. However, the relationship of the filamentous appendage to the capsule is still not clear. There are two possible explanations of the results. Either the filaments are true filamentous organelles present in the living organism or they are artifacts produced during the fixation or dehydration of the capsule. There is some evidence against the possibility of artifacts. Filamentous appendages of a comparable diameter were shown after four different methods of preparation (Figs. 6.12-6.15). Although dehydration of the capsule could lead to the formation of filaments it is doubtful that such regularity in form and thickness could be achieved by drying.

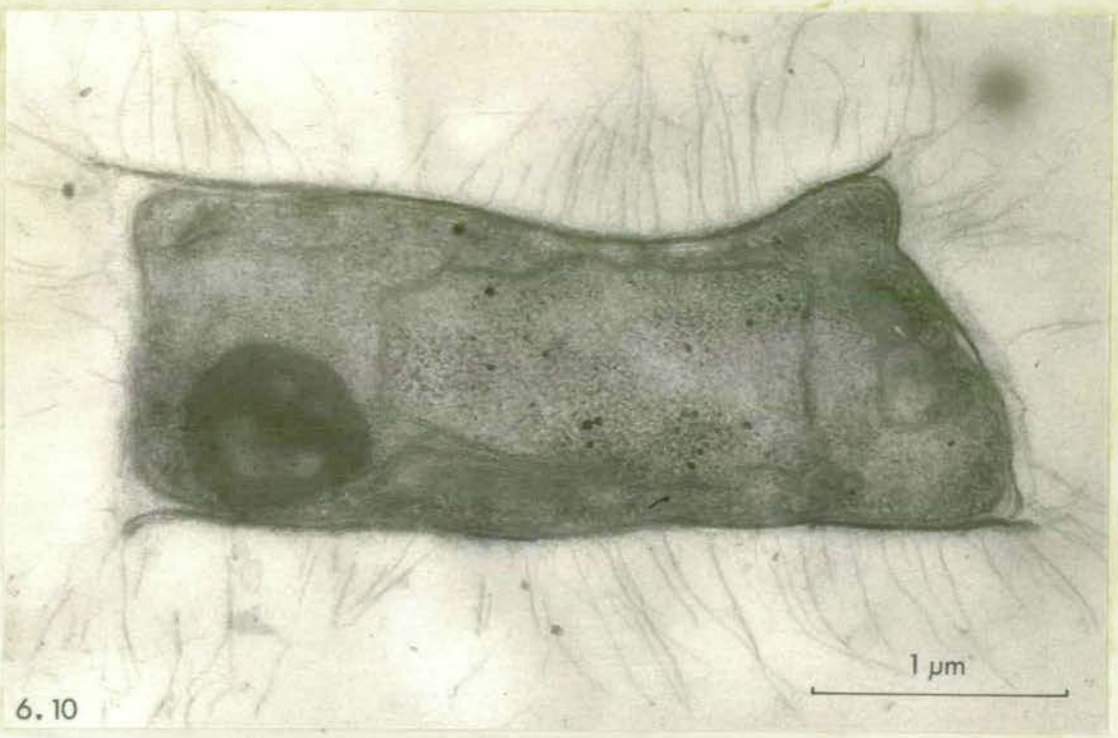
At the time when this study was started there was no published account of the fine structure of oidia. Observations on the ultrastructure of oidia of Coprinus lagopus (= C.cinereus) have since been published by Heintz and Niederpruem (1971). There is however no indication of the presence of a capsule or filamentous appendages in their account. The shape of oidia of Coprinus cinereus is similar to that of

P.coprophila. The flat ends also have terminal flanges. There are also similarities in the internal structure. The nucleus, mitochondria, ribosomes and lipid droplets are similar in shape and position. However the glycogen granules and the electron-dense pleomorphic inclusions which they described are absent from P.coprophila preparations.

The filamentous appendages described in this study have not before been found on oidia, but have been reported in other microorganisms. The term 'fimbriae' which was originally used to describe rigid, hair-like appendages on the surface of many capsulate and non-capsulate bacteria (Duguid et al, 1955) has now been broadened to include branched and flexuous filaments, (Yanagava and Otsuki, 1970; Wistriech and Baker, 1971).

Recently Poon and Day (1974) reported fimbriae in the sporidial cells of Ustilago violacea. The metal shadowed preparations showed fimbriae which are curved and flexible and of approximately the same dimensions as the oidial filaments. However these fimbriae were not stained by either phosphotungstic acid or ruthenium red.

Bacterial fimbriae are usually made of single proteins and often possess antigenic properties. The chemical nature of the oidial filaments is not yet known and further work is necessary before more certain interpretations of this work can be made.



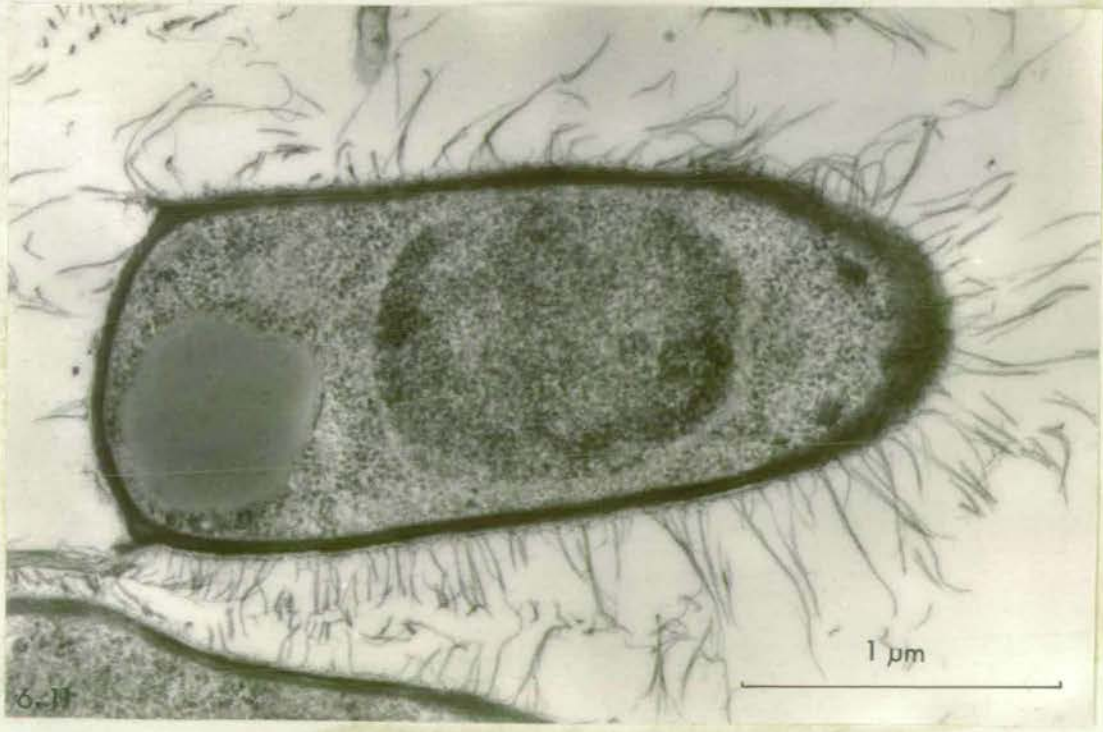
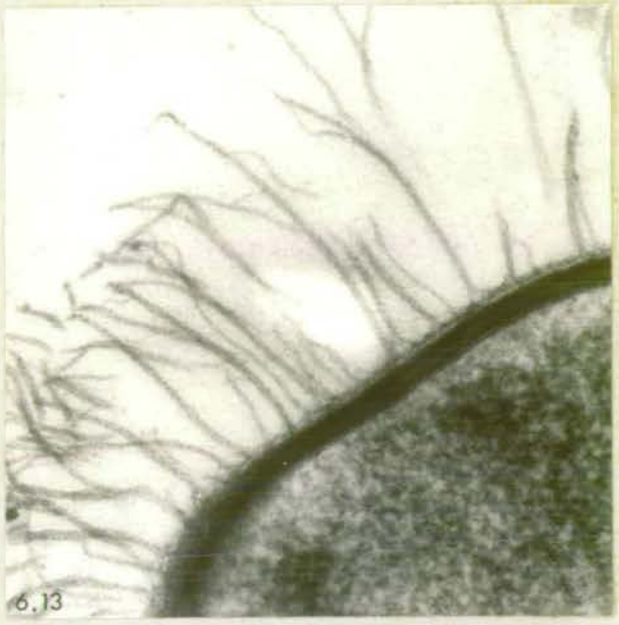
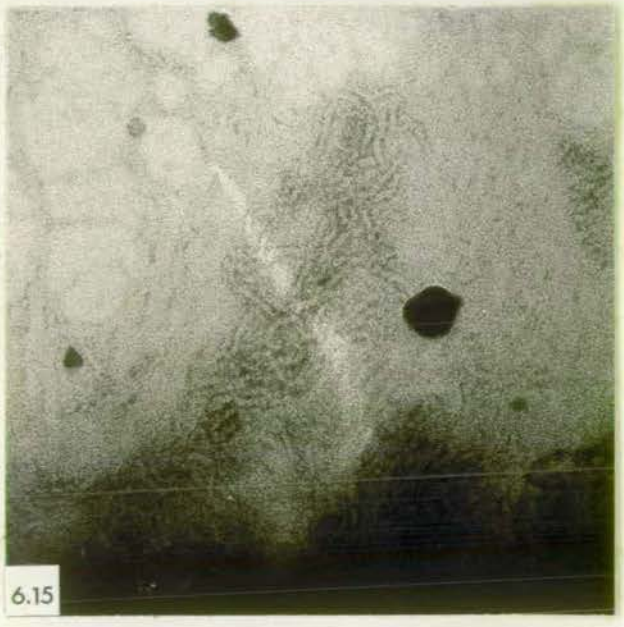


Fig. 6.10 Electron micrograph of a longitudinal section of an oidium fixed in osmic acid only (method i) showing filaments.

Fig. 6.11 Electron micrograph of a longitudinal section of an oidium double fixed in glutaraldehyde and osmic acid (method ii) showing filaments.





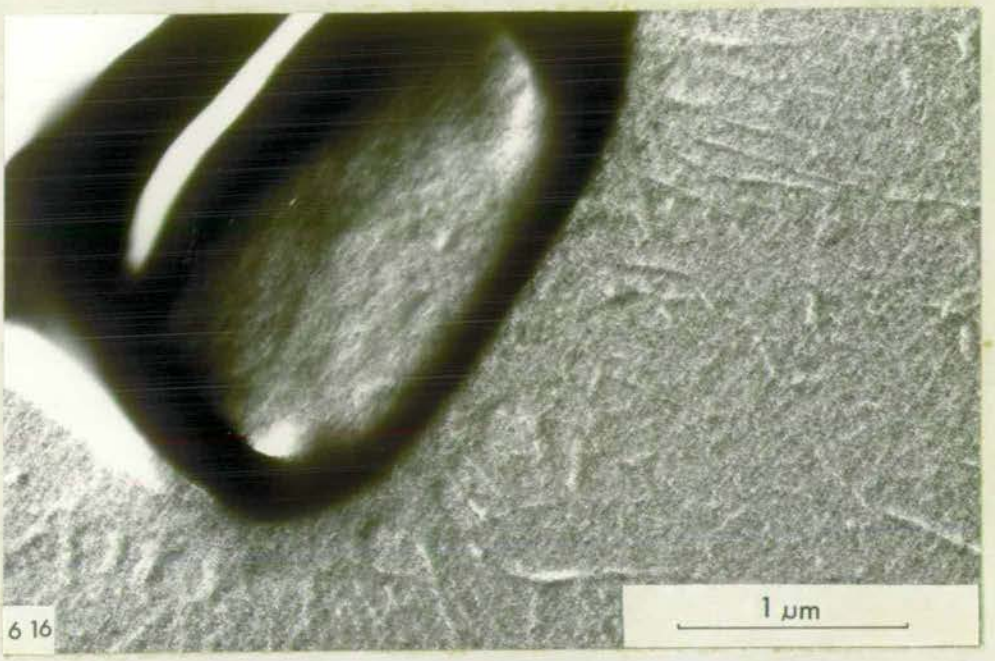
Electron micrographs of filaments after four different methods of preparation. 2 μm

Fig.6.12 Filaments on sectioned oidia fixed in osmic acid only (method i).

Fig.6.13 Filaments on sectioned oidia fixed in glutaraldehyde and osmic acid (method ii).

Fig.6.14 Filaments on sectioned oidia fixed in glutaraldehyde and osmic acid in the presence of ruthenium red (method iii).

Fig.6.15 Filaments on whole oidia negatively stained with 3% phosphotungstic acid (method iv).



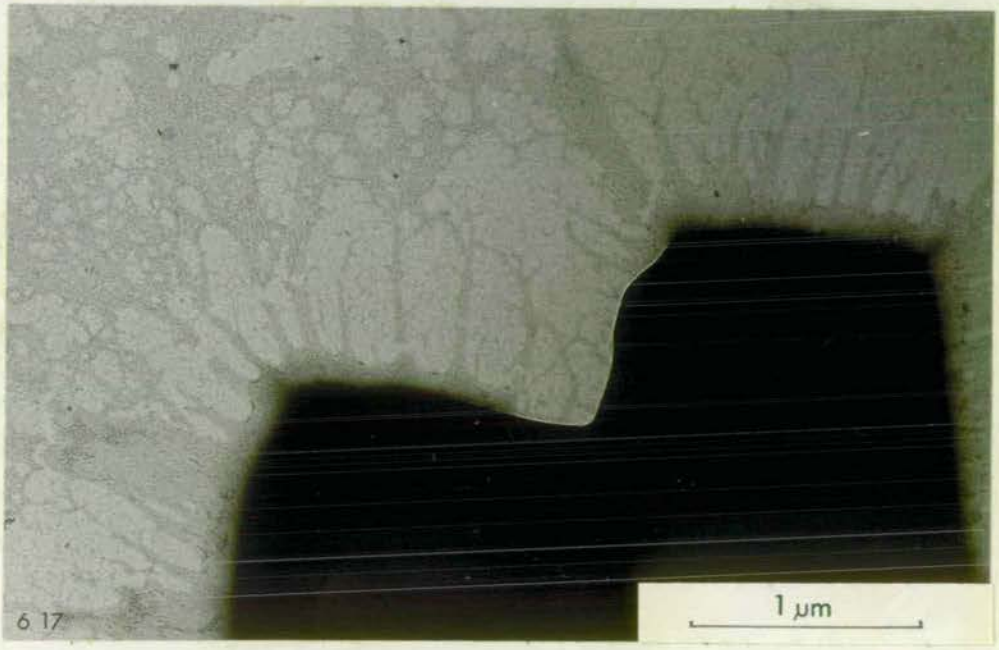
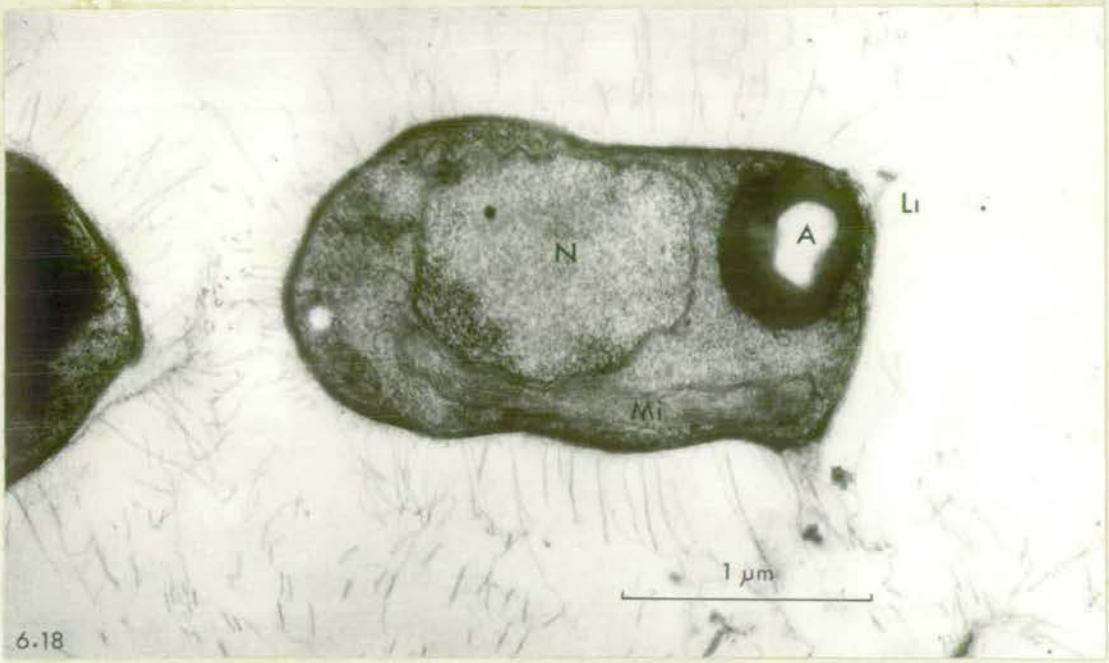


Fig. 6.16 Electron micrograph of oidial surface after metal shadowing (method v).

Fig. 6.17 Electron micrograph of oidial surface after negative staining (method iv).



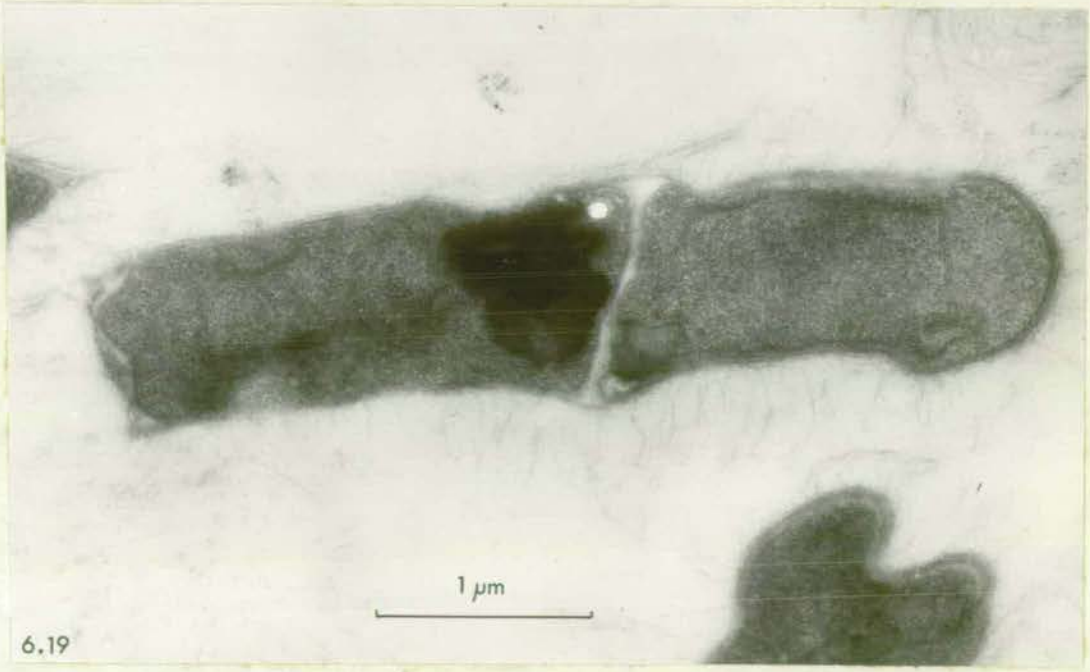


Fig. 6.18 Electron micrograph of an oidium fixed in osmic acid only (method i) showing nucleus (N), mitochondrion (Mi), and lipid droplet (Li) with artifact (A).

Fig. 6.19 Electron micrograph of two unseparated oidia fixed in osmic acid only (method i).

CHAPTER 7 : THE HOMING OF HYPHAE TOWARDS OIDIA

7.1 Introduction

Buller (1933) observed that hyphae are often attracted towards each other and was intrigued by the physiological processes which were involved. The observation that hyphae are also attracted to oidia was made only recently by Kemp (1970) and Bistis (1970). The directed growth or 'homing' can be clearly seen under the low power of the microscope and the whole test can be completed within an hour.

In the original short note Kemp (1970) reported the occurrence of homing in a number of Coprinus species, Flammulina velutipes, and Psathyrella stercorea (= P.coprophila Breeding Group I, Isolate 1). Both monokaryotic and dikaryotic hyphae were found to react to the oidial stimulus but the monokaryotic hyphae reacted more readily. In all isolates tested the hyphae homed towards their own oidia regardless of mating type, while most interspecific combinations failed to have any effect. However when the oidia of Coprinus congregatus were tested against the hyphae of C.bisporus the homing and fusion was followed by the vacuolation and death of the hybrid cell. From this it was interpreted that the two species are distinct but closely related. Kemp concluded that homing followed by dikaryotisation is a species specific reaction and pointed out the usefulness of the method in distinguishing between different species.

Brown (1971) observed the sequence of events which takes place in the course of fusion of hyphae with oidia in Flammulina velutipes. With the help of a micromanipulator he placed an oidium within varying distances from the hyphal tip and noted the time of response. He concluded that the reaction was usually complete within 30 minutes and that

the hyphae were not stimulated from distances greater than 20 µm.

In the present study homing was used extensively to test the degree of affinity between related isolates. These results and the taxonomic conclusions are given in Chapters 8, 9 and 10. In this chapter are described some general observations on the homing of hyphae towards oidia.

7.2 Method of observing the homing reaction

Monokaryotic mycelia were always used since they were found to react more strongly to the oidial stimulus. Two vigorously growing mycelia, at least one of which was actively producing oidia were required at the same time. If both mycelia produced oidia, reciprocal tests were made. A droplet of sterile water held in a flamed tungsten loop was used to transfer the oidia from one culture to another. The water droplets formed by condensation on the inside of the petri dish lid were useful as a source of sterile water. The loopful of oidia was smeared along the edge of a growing monokaryotic colony. Contamination with oidia belonging to the resident colony was avoided by placing the droplet clear of the resident colony periphery. A number of oidial droplets could be smeared around the same colony. The water in the smeared droplets was quickly absorbed into the agar leaving a deposit of oidia. The plate was then covered and left at room temperature.

The reaction of the hyphae on reaching the oidial smear was observed directly on agar under the low power of a Vickers Patholux microscope. Two observations were made, the first about an hour after smearing and the second 3-6 hours later. At the second observation any evidence of vacuolation was noted.

7.3 Results

7.3.1 Description of the homing reaction

Photographs of a positive homing reaction are shown in Figs. 7.3-7.5. Photographs of a positive homing reaction followed by vacuolation and death of the fusion cell are shown in Figs. 7.6-7.10. The sequence of events leading to the fusion is the same regardless of the events which follow.

The oidial stimulus causes a change in the direction of hyphal growth and the hyphal tip may curl round towards the oidium (Fig. 7.1a). The lateral proximity of an oidium to a growing hypha sometimes acts as a stimulus for the formation of a side branch. The side branch appears as a short peg and then rapidly curls round and fuses with the oidium (Fig.7.1b and 7.4).

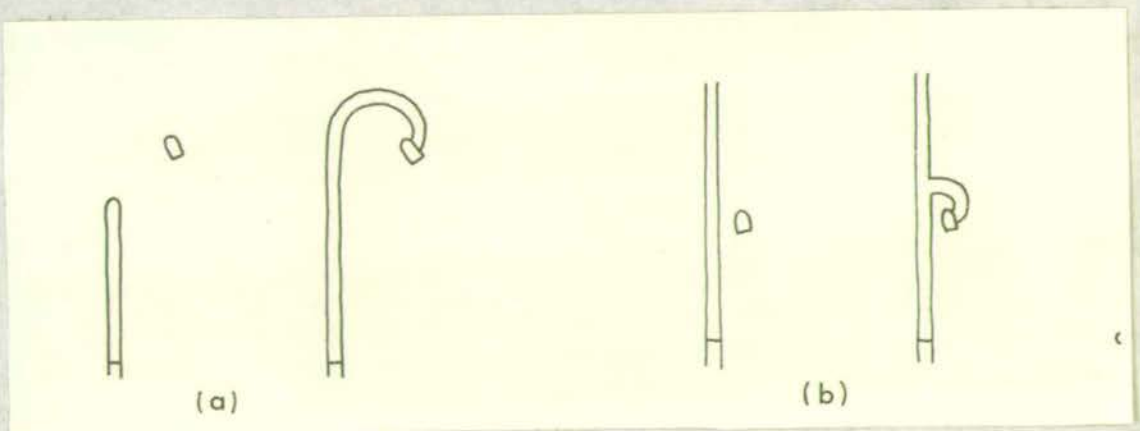


Fig.7.1 The homing of hyphae towards oidia
(a) oidial stimulus at the hyphal tip
(b) oidial stimulus lateral

The homing of a hyphal tip is usually complete within 15-30 minutes. Within an hour many fusions can be observed at the periphery of the colony and a positive homing reaction can then be convincingly scored.

A positive homing reaction takes place even if the hyphae and oidia are separated by a layer of cellophane. When a droplet of oidial suspension was placed on a piece of sterile wettable cellophane (2 inch^2) over a mycelial colony, the hyphal tips grew towards the oidia even though fusion could not take place. In a control experiment in which oidia were not placed on the cellophane, there was no appreciable change in the direction of hyphal growth. The active chemotropic substance is only produced by relatively fresh oidia. When oidia, one to two months old were used, no reaction was observed.

7.3.2 Homing of hyphae towards oidia of the same isolate

A positive reaction was observed in all tests involving different monokaryons of the same isolate. A list of the isolates tested is given below.

P.candolleana Breeding Group I	: Isolates 141,145,169,170,173,175,176,179.
P.candolleana Breeding Group II	: Isolates 136,142,146,171.
P.candolleana Breeding Group III	: Isolate 300.
P.candolleana Breeding Group IV	: Isolate 178.
P.coprophila Breeding Group I	: Isolates 1,51,84,128,129.
P.coprophila Breeding Group II	: Isolate 59.
P.gossypina	: Isolate 106.
P.gracilis Breeding Group I	: Isolates 3,55,58,110,111.
P.gracilis Breeding Group II	: Isolates 121,151.
P.gracilis Breeding Group III	: Isolates 72,101,104.
P.obtusata	: Isolate 5.
P.orbitarum	: Isolate 25.
P.spadiceo-grisea	: Isolate 8.
P.vernalis	: Isolate 18.

In Isolates 84 and 59 the four mating types were tested against one another. When oidia belonging to mating types A1B1, A2B2, A1B2, and A2B1 were tested in all combinations against the mycelia there was an equal response in each of the tests. The experiment showed that homing, at least in these isolates, is independent of mating type. If the oidia were of compatible mating type, fusion was usually followed by migration and division of the oidial nucleus and dikaryotisation of the resident mycelium.

7.3.3 Homing of hyphae towards oidia of different isolates

The following three responses were observed between hyphae and oidia of different isolates:

1) no homing, no fusion

2) homing, fusion, lethal reaction

3) homing, fusion, dikaryotisation

Reciprocal tests usually gave the same results.

Reaction (3) leads to clamp formation and the migration of nuclei with the oidial genotype indicating that the hyphae and oidium belong to the same breeding group. Reactions (1) and (2) indicate two levels of relationship above the breeding group. The rate and frequency of homing is similar to the response obtained when the test involves members of the same breeding group.

Examples of the lethal reaction are shown in Figs. 7.5-7.10. The photographs show that vacuolation appears to start at the distal end of the fusion cell and to proceed in both directions. The lethal reaction affects not only the fusion cell but also the adjacent cells (Fig.7.10). Similar lethal reactions have been observed following the fusion of monokaryotic hyphae. However, hyphal homing is much easier to detect than hyphal anastomosis.

7.4 Discussion

The observations described in this chapter show that a study of the response of hyphae to oidia can be used to indicate the affinity of isolates. In this study reciprocal tests usually gave the same results and therefore only three types of responses were found. Kemp (unpublished) found in Coprinus that the reactions in reciprocal tests were often different, and so recognised six possible pairs of responses (Table 7.1).

Table 7.1 The six different types of response between hyphae and oidia of two hypothetical isolates A and B (after Kemp, unpublished)

	Oidia of A on B	Oidia of B on A
1	Homing, lethal	Homing, lethal
2	Homing	Homing, lethal
3	Nil	Homing, lethal
4	Homing	Homing
5	Nil	Homing
6	Nil	Nil

Lethal reactions similar to those described in this chapter have been described by other authors in other fungal genera. Flentje and Stretton (1964) described a similar range of reactions between the hyphae of different isolates of Thanatephorus cucumeris and T.praticolus. No mutual attraction between the hyphae was observed but in some combinations of isolates the anastomosed cells collapsed and died. In some cases up to six cells on either side were affected which suggests that the antagonistic substance was able to cross through the cell septa. This was also noticed in the lethal reaction described in the present study.

Carlile and Dee (1967) and Carlile (1972) observed a lethal reaction following plasmodial fusion between two strains of the myxomycete Physarium polycephalum. Some combinations of strains fused completely so that within a few days it was no longer evident that the resulting plasmodium originated from two separate plasmodia. Other combinations of strains consistently failed to fuse. With yet other combinations fusion took place but was followed by a destructive reaction resulting in a cessation of streaming, gelling of the cytoplasm and changes in colour and appearance of the area at or near the region where fusion occurred.

Ikediugwu and Webster (1970) reported a 'hyphal interference' reaction following contact between the hyphae of some unrelated coprophilous species. Vacuolation, loss of opacity and hydrostatic pressure took place though there was no cell fusion. The antagonistic substance probably diffused through the hyphal walls. When mounted in neutral red the affected cells were found to take up the red stain. Neutral red was tried in this study in an attempt to differentiate between the damaged and healthy cells but caused the bursting of hyphal tips in both.

Two substances or groups of substances of unknown chemical composition appear to be involved in the homing and lethal reactions which are described in this chapter. The substance or substances responsible for hyphal stimulation are produced only by viable oidia, are clearly chemotropic and are capable of diffusing over short distances (20 µm) through cellophane and agar. Bistis (unpublished) suggested that the stimulating substance is unstable and short lived. There is a possibility that the filamentous appendages described in the previous

chapter are responsible for the diffusion of the active substance out of the oidium. Both 'wet' and 'dry' oidia are known to stimulate hyphae so the presence of a mucilagenous capsule is clearly not essential. The substance or substances responsible for the lethal reaction do not diffuse through agar but are formed following the fusion of two antagonistic cytoplasms. The antagonistic agent appears to be able to pass across the cell septa into the adjacent cytoplasm.

Fig. 7.2 Germinating oidia of P.candolleana (Isolate 142). (x 590)

Fig. 7.3 The homing of hyphae towards oidia. The hyphae and oidia are derived from the same isolate (No.59). (x 190)

Fig. 7.4 Normal homing of hyphae towards oidia showing the curving of hyphal tips. The hyphae belong to Isolate 146 and the oidia to Isolate 136. Both isolates belong to P.candolleana Breeding Group II. (x 375)

Fig. 7.5 Normal homing of hyphae towards oidia showing fusion by peg formation. The hyphae belong to Isolate 145 and the oidia to Isolate 141. Both isolates belong to P.candolleana Breeding Group I. (x 375)

Fig. 7.6 (a) Homing of hyphae of Isolate 55 (P.gracilis Breeding Group I) towards oidia of Isolate 101 (P.gracilis Breeding Group III) (x 590)

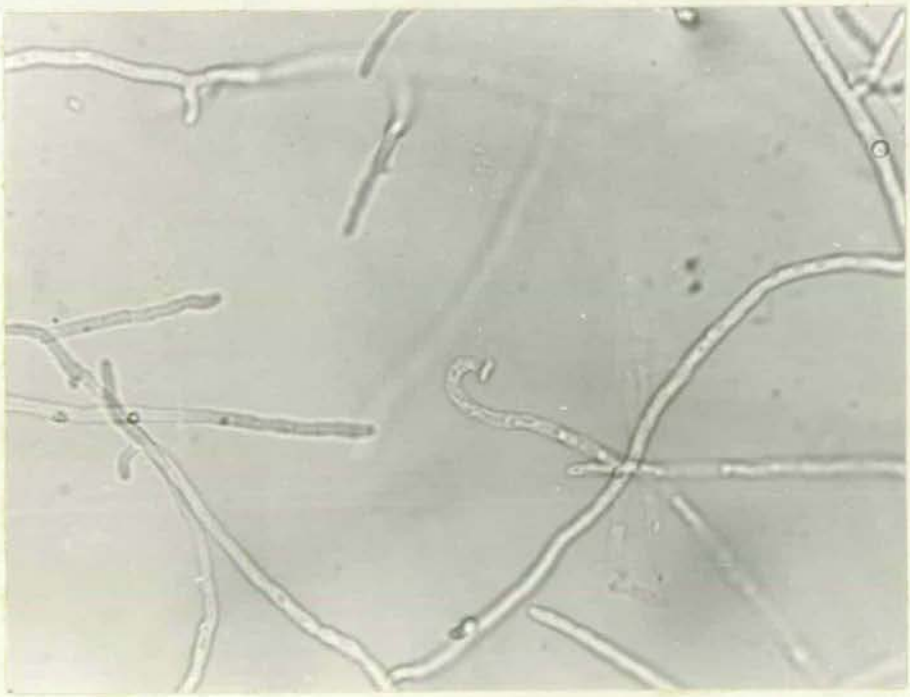


Fig.7.6 (b) The same hyphal tip 2 hours later showing lethal reaction

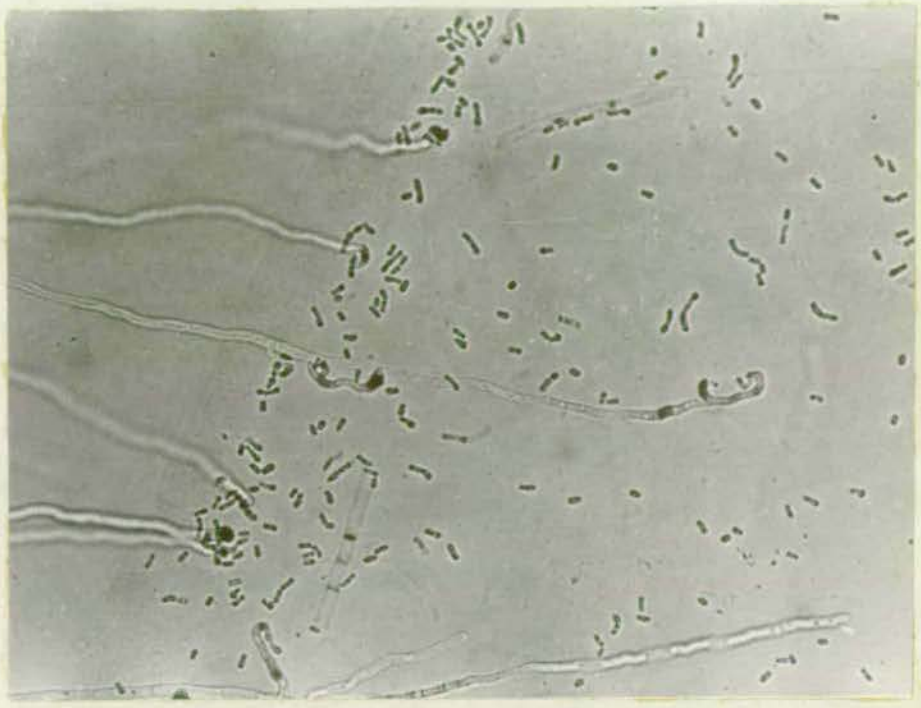


Fig. 7.7 Lethal reaction showing vacuolation of hyphal cells following homing and fusion between hyphae of Isolate 145 (P.candolleana Breeding Group I) and oidia of Isolate 142 (P.candolleana Breeding Group II). (x 375)

Fig. 7.8 Lethal reaction showing vacuolation of hyphal tips following homing and fusion of hyphae of Isolate 142 (P.candolleana Breeding Group II) and oidia of Isolate 145 (P.candolleana Breeding Group I). (x 375)

Fig. 7.9 Lethal reaction showing homing and vacuolation of the terminal hyphal cell. The hyphae belong to Isolate 145 (P.candolleana Breeding Group I) and the oidia to Isolate 142 (P.candolleana Breeding Group II). (x 375)

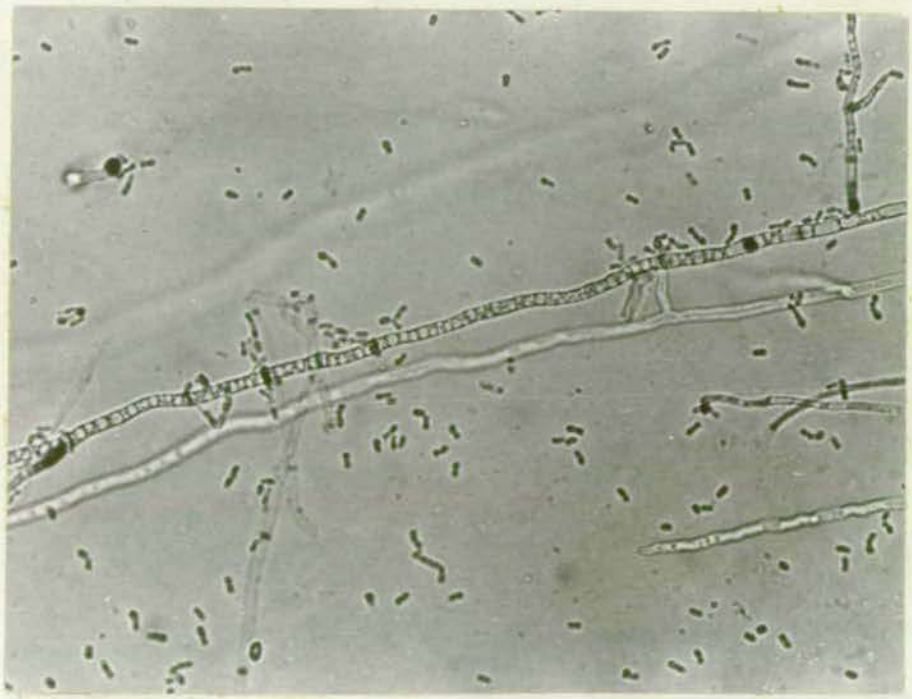


Fig. 7.10 Lethal reaction showing vacuolation of intercalary hyphal cells. The hyphae belong to Isolate 145 (P.candolleana Breeding Group I) and the oidia to Isolate 142 (P.candolleana Breeding Group II). (x 375)

CHAPTER 8 : STUDIES IN THE P.GRACILIS GROUP OF ISOLATES

8.1 Introduction

P.gracilis is one of the most common and variable Psathyrella species. Although it is fairly easy to place isolates in this morphological complex using a few easily defined characters such as pink gill edge and rooting base, it is difficult to further divide the complex into species. Two entirely different approaches were chosen by van Waveren (1971a) and Smith (1972). Van Waveren, in his study of European isolates divided the complex into two species P.gracilis (Fr.)Quel. and P.microrrhiza (Lasch.) Konr. & Maubl. P.gracilis was further divided into 5 formae. Smith in his study of North American isolates did not acknowledge the existence of a superspecific category equivalent to the P.gracilis complex, but dedcribed 12 species with a pink gill edge which appear to be scattered through several subsections and series of the section Psathyrella. The morphological variability of this group and the discrepancy in taxonomic treatment by these two Psathyrella specialists, made it worthwhile to investigate the group biosystematically.

28 isolates were collected from nature mostly by myself. The fruit-bodies were described morphologically and cultures were established by the germination of basidiospores. Monokaryotic mycelia were crossed in order to establish breeding groups and homing responses were studied in order to determine further levels of relationship. Then by comparing the morphological characters within and between the breeding groups the constancy of such morphological characters on which taxonomic units are usually based was assessed. The taxonomic status and the possible evolution of the breeding groups is here discussed, and the biological validity of the species concepts of van Waveren and Smith is compared.

8.2 Materials and Methods

The 28 isolates which were used in this study are listed in Table 8.1. The isolates were initially placed in the L.gracilis complex using the following group of characters.

1. Pink tinges on the cap, especially on drying.
2. Pink edge on the gills.
3. Rooting base.
4. Lack of macroscopically distinct veil.
5. Abundant cystidia on the gill sides and faces.
6. Spore print purplish in a thin layer, vinaceous-black in a thicker layer.

All the isolates were collected from a similar habitat. This was typically a roadside grass verge or an infrequently mown rough grass lawn or meadow. The high free nitrate content of the substrate was indicated by the frequent presence of nettles. Also one of the best collecting sites was the grounds surrounding a crematorium. The rooting base, where present, was often attached to small twigs on or below the surface of the ground.

The fruit-bodies were brought to the laboratory as soon as possible after collection and were examined morphologically. Herbarium material was preserved and in some cases was examined later. Monokaryotic mycelia were established as described in Section 2.4. The basidiospores were heat treated in order to improve the percentage germination (Chapter 3).

Crosses between monokaryotic mycelia of different isolates were made four per plate, placing the inocula about 8 mm apart. The inocula were placed further apart than in the mating experiments so as to give each inoculum time to establish itself in case of possible differences in growth rate. A few days after the mycelia had met subcultures from the junction line were taken and incubated for two days. They were then examined micro-

Table 8.1 List of isolates in the P.gracilis complex.

Isolate No.	Place of collection	Map Grid reference	Month and Year of collection	Collector
3	Edinburgh, Scotland	NT 184 773	Aug. 1969	M.J.
15	Midlothian, Scotland	NT 217 583	Sept. 1969	M.J.
16	Edinburgh, Scotland	NT 18- 76-	Oct. 1969	M.R.
17	Edinburgh, Scotland	NT 184 773	Oct. 1969	M.J.
55	Perthshire, Scotland	NO 057 627	Aug. 1970	M.J.
58	Perthshire, Scotland	NO 052 629	Aug. 1970	M.J.
60	Perthshire, Scotland	NO 050 631	Aug. 1970	M.J.
67	Edinburgh, Scotland	NT 185 773	Sept. 1970	M.J.
68	Edinburgh, Scotland	NT 185 773	Sept. 1970	M.J.
70	Edinburgh, Scotland	NT 184 773	Sept. 1970	M.J.
71	Edinburgh, Scotland	NT 185 773	Sept. 1970	M.J.
72	Edinburgh, Scotland	NT 270 683	Oct. 1970	M.J.
73	Edinburgh, Scotland	NT 270 683	Oct. 1970	M.J.
74	Edinburgh, Scotland	NT 270 683	Oct. 1970	M.J.
101	Yorkshire, England	SE 554 400	Sept. 1971	M.J.
104	Falkirk, Scotland	NS 963 784	Oct. 1971	M.J.
105	Falkirk, Scotland	NS 962 768	Oct. 1971	M.J.
110	Midlothian, Scotland	NT 375 685	Oct. 1971	M.J.
111	East Lothian, Scotland	NT 419 681	Oct. 1971	M.J.
112	East Lothian, Scotland	NT 505 682	Oct. 1971	M.J.
117	Moray, Inverness-shire	-	Oct. 1971	G.S.
121	Devon, England	SX 916 815	Oct. 1971	M.J.
151	Warwickshire, England	SP 083 824	1971	S.P.620
152	Delden, Holland	-	Oct. 1971	K.vW.30*
153	Delden, Holland	-	Oct. 1971	K.vW.23*
156	Warwickshire, England	-	1971	S.P.658
157	Warwickshire, England	SP 244 333	1971	S.P.649
158	Warwickshire, England	-	1971	S.P.664

* isolates identified as P.microrrhiza by sender

Abbreviations: M.J. - myself; M.R. - Michael Richardson; G.S. - George Shepherd; S.P. - Stanley Porter; K.vW. - Kits van Waveren.

scopically for the presence of clamp connections. In most cases clamps were looked for only at the junction line, since the importance of nuclear migration information in mycelial mating and crossing was realised late in the study. If clampy dikaryons were found it was assumed that the monokaryons could exchange genetic material and that they therefore belong to the same breeding group. The occurrence of aversion lines between the crossed mycelia was noted. Homing responses were studied as described in Section 7.2.

8.3 The Breeding Groups

Mycelia of the 28 isolates were crossed in most combinations and three breeding groups were found (Fig.8.1). Although nuclear migration was not studied in most crosses, this information is not as important in the mycelial crossing experiments as it is when mycelia from the same isolate are mated. In crosses which were made later in the study information on nuclear migration was noted and in Breeding Groups I and II bilateral or unilateral migration was usually found. In crosses between different isolates of Breeding Group III nuclear migration was never observed. The failure of nuclear migration in matings within isolates of Group III is discussed in Section 4.4.2.

Aversion lines were observed in all crosses involving isolates of different breeding groups from about 5 days after the meeting of mycelia. In crosses between Breeding Groups I x II and II x III the aversion lines were more prominent than those in crosses between Breeding Groups I x III.

The results of homing tests between some of the isolates are shown on Fig. 8.2. Within each of the three breeding groups positive homing responses were observed. A positive homing response was also observed

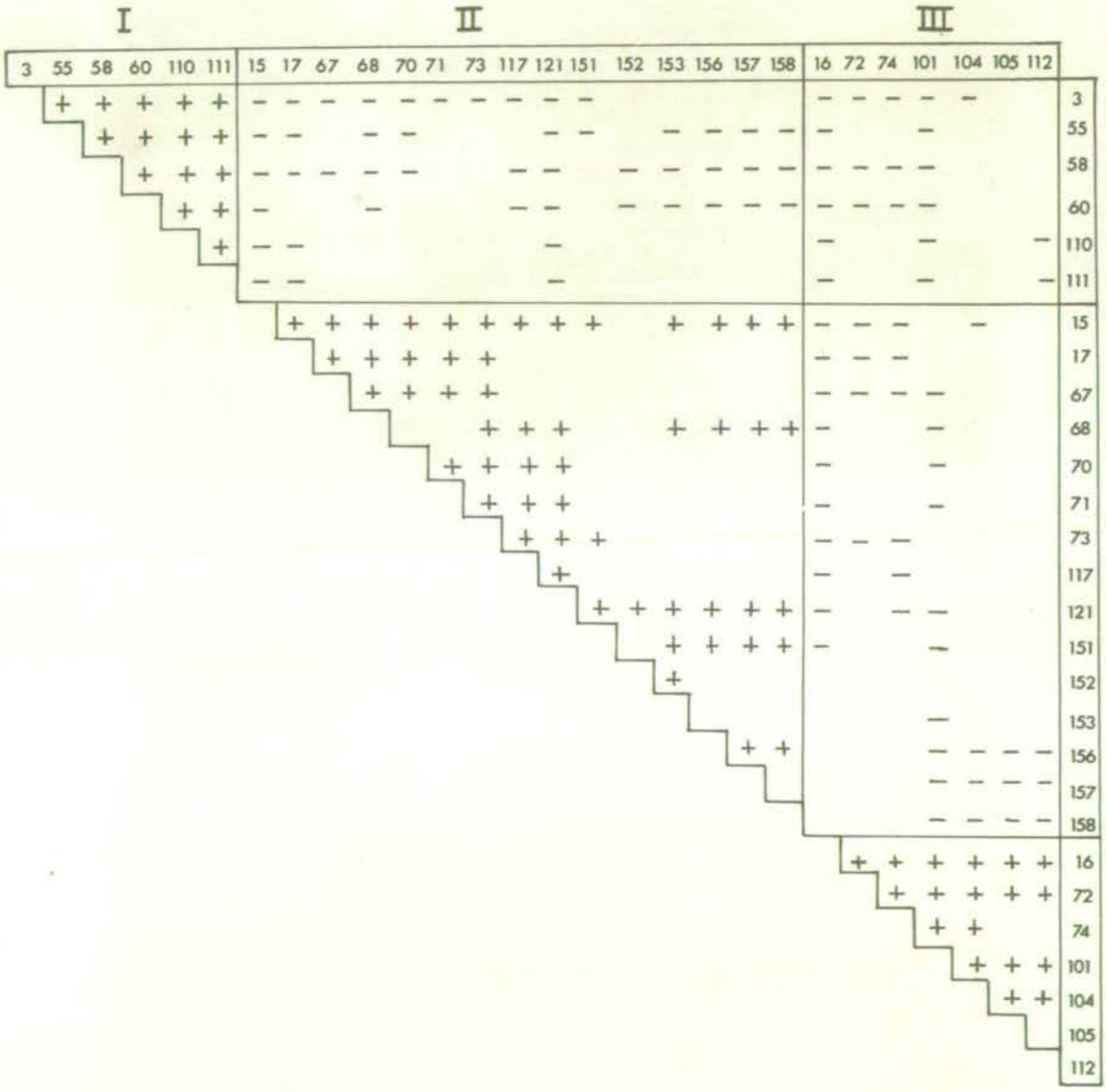


Fig. 8.1 Results of mycelial crossing in the P.gracilis group of isolates.

\+ dikaryon with clamps formed
\- dikaryon with clamps not formed

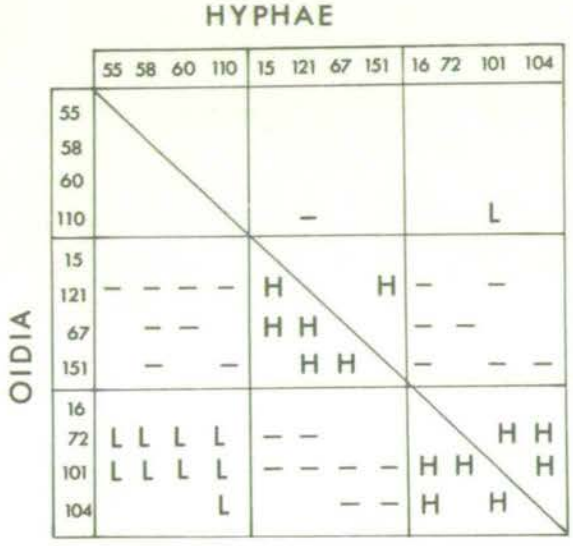


Fig. 8.2 Homing responses between selected isolates in the P.gracilis group of isolates.

H Homing of hyphae towards oidia

L Homing of hyphae towards oidia followed by a lethal reaction

– No homing response

between hyphae of Breeding Group I and oidia of Breeding Group III, but this was followed by the vacuolation and death of the hybrid cell. An example of such a lethal reaction has been photographed and is shown on Fig. 7.6 b. The reciprocal test between the hyphae of Breeding Group III and oidia of Breeding Group I was done only once and a lethal reaction was also observed. It was not possible to repeat the test since mycelia of Breeding Group I produced oidia very infrequently.

8.4 Morphological comparisons

Some morphological comparisons were made between the groups to find if the breeding groups could be distinguished morphologically, and within the groups to assess the variability of the characters. The characters which were examined are commonly used by taxonomists as a basis for the description of taxonomic units.

8.4.1 Macroscopic characters of the fruit-body

The following characters were examined.

1. Cap diameter
2. Stipe length
3. Stipe breadth
4. Length of the rooting base, if present
5. Cap colour when moist
6. Cap colour when dry
7. Colour of gill face
8. Colour of gill edge

Habit drawings of several isolates are shown on Fig.8.3. Some measurements are given on Table 8.2. The colour of the fruit-bodies is described on Table 8.3. The colour chart of Henderson, Orton and Watling (1970) was used.

It can be seen from the measurements on Table 8.2 that the fruit-

Fig. 8.3 Habit diagrams of some isolates of the P.gracilis complex. Isolate Nos. are indicated with the Arabic numbers and the breeding groups with the Roman numerals. (Life size)

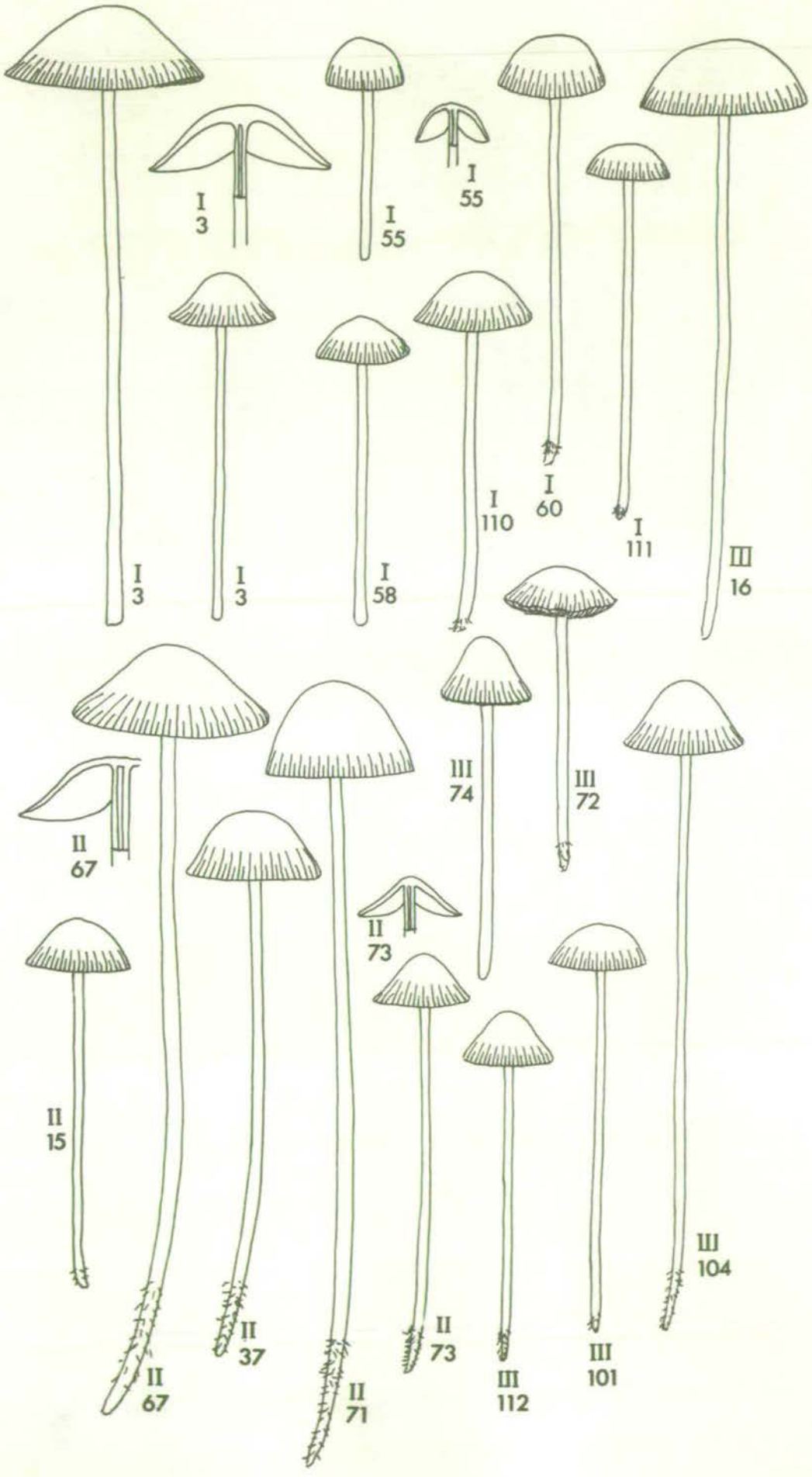

I 3
I 55
I 55
I 3
I 3
I 58
I 110
I 60
I 111
III 16
II 67
III 74
III 72
II 73
II 15
II 37
II 67
II 71
II 73
III 112
III 101
III 104

Table 8.2 Fruit-body measurements in the P.gracilis group of isolates. More than one measurement is given where several fruit bodies were found in a clump.

Isolate No.		Cap Diameter (mm)	Stipe		Rooting base (mm)
			Length (mm)	Breadth (mm)	
I	3	15, 30	50, 90	2.0	none found
	55	9, 12	28	1.5	none found
	58	16	45	1.5	very slight
	60	18, 65	20, 65	1.5, 2.0	very slight
	110	20	53	1.5	very slight
	111	14	57	2.0	very slight
II	17	20, 40	60	2.0, 3.0	30
	67	12, 38	120, 150	1.5, 3.0	20
	68	30	110	2.0	15
	71	25	110	1.5	15
	73	16, 20	50, 80	1.5	20
III	16	27	90	not measured	none found
	72	18	30	2.0	very slight
	74	14	45	1.5	none found
	101	15	65	1.5	very slight
	104	20	90	1.5	10
	105	15	62	2.0	very slight
	112	11, 14, 32	33, 43, 55	1.5, 2.0, 3.0	15

Table 8.3 Cap and gill colour in fruit-bodies of the P.gracilis group of isolates.

	Isolate No.	Cap Colour		Gill Colour	
		Moist	Dry	Gill face	Gill edge
I	3	vinaceous-buff	pinkish-grey	sepia	pink
	55	vinaceous-buff	vinaceous-buff	mouse-grey	pink
	58	vinaceous-buff	pinkish-grey	mouse-grey	pink
	60	vinaceous-buff	pinkish-grey	mouse-grey	pink
	110	vinaceous-buff	pinkish-buff	sepia	pink
	111	buff	buff	sepia	white
II	17	clay-buff	yellowish	mouse-grey	brown
	67	clay-buff	pinkish	mouse-grey	pink
	71	vinaceous-buff	pink	mouse-grey	pink
	73	buff	buff	mouse-grey	pink
III	16	pale-buff	pale-buff	-	pink
	72	buff	pinkish	vinaceous-grey	pink
	101	sepia	pinkish	mouse-grey	pink
	104	vinaceous-buff	pinkish	mouse-grey	pink
	105	vinaceous-buff	pinkish	dark-grey	pink
	112	vinaceous-buff	pinkish	mouse-grey	pink

bodies of Breeding Group II are in general more robust than those of Breeding Groups I and III and that they have a longer rooting base. In Breeding Group I the rooting base is either absent or very fragile, while in Breeding Group III it is intermediate in size. However, there is a considerable overlap.

No obvious differences in cap colour were found between the groups. The pink tinges on the cap and gill edge were common to most isolates of all three groups. Only Isolates 111 and 37 had no pink tinge on the cap or gill. Even this feature which is considered to be diagnostic of the P.gracilis complex probably depends on the environmental conditions.

To conclude, fruit-body habit and colour may be used to identify isolates to the P.gracilis complex, but though there are some differences, it is difficult to use these characters to distinguish between the breeding groups.

8.4.2 Basidiospores

The following characters were examined.

1. Spore print colour
2. Basidiospore length
3. Basidiospore width
4. Ratio of basidiospore length/width
5. Spore shape
6. Spore colour under the microscope

Spore prints were made from fresh cap material soon after collection. The spore prints of all isolates were purplish-grey in a thin layer and vinaceous-black in a thicker layer and no difference between the breeding groups were noted. Spores of 14 isolates were further examined microscopically. Spores from spore prints were used whenever possible. They were mounted in gum chloral and examined using an oil

immersion objective. Gum chloral causes neither shrinkage nor swelling of basidiospores (Watling, 1964) and so is a good mounting medium. Outlines of spores were traced on paper using a Camera Lucida, and spore length and width measurements were made with a ruler. At least 20 spores were measured per isolate. The measurements were converted to μm to the accuracy of one decimal place. This accuracy is slightly artificial since the resolving power of the microscope used is only 0.2 μm. Spores were drawn in face view or profile depending on the way they were lying on the slide. The short diameter was found to be approximately the same both in face view and profile, and so no distinction was made between these positions in the calculation of the means. The ratio of spore length to width was calculated since Corner (1947) considered it to be the best expression of spore size. The means and ranges of length, width, and ratio (R) were calculated. The results for 14 isolates are set out in Table 8.4. One average sized basidiospore from each of 14 isolates is drawn on Fig.8.4.

Table 8.4 shows that, with the exception of Isolate 15, isolates belonging to Breeding Group II have, on average, smaller spores than the remaining isolates. The 140 spore length measurements of Breeding Group II (without Isolate 15) and the 120 pooled length measurements of Breeding Groups I and III were compared using the Student's t test and the difference between the means was found to be statistically significant. The individual spore length measurements are listed in Appendix III. Even though a significant difference was found it was difficult to use spore size to distinguish between the breeding groups since the size ranges of the individual isolates showed a considerable overlap. Isolate 15 was

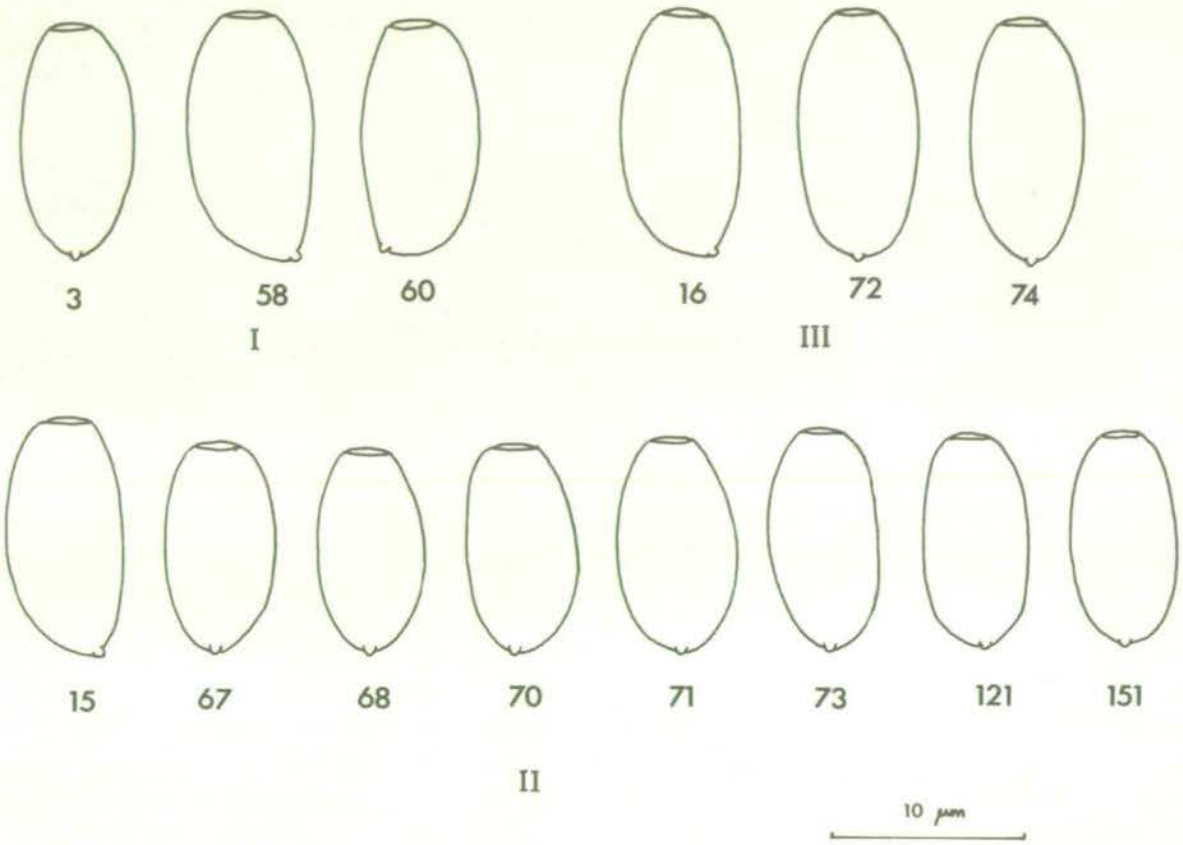


Fig. 8.4 Average basidiospores in the P.gracilis complex. Isolate numbers are indicated with the Arabic numbers and the breeding groups with the Roman numerals.

Table 8.4 Basidiospore measurements in the P.gracilis group of isolates

	Isolate No.	Length		Width		Length/Width Ratio	
		Mean (µm)	Range (µm)	Mean (µm)	Range (µm)	Mean (µm)	Range (µm)
I	3	12.7	11.5-13.6	6.3	5.6-6.6	2.03	1.86-2.26
	58	13.3	12.0-15.1	6.8	6.2-7.2	1.95	1.71-2.32
	60	12.6	11.8-13.4	6.4	6.1-6.9	1.95	1.77-2.13
II	15	13.1	11.4-15.1	6.3	5.9-7.5	2.05	1.87-2.23
	67	11.5	10.8-12.0	6.3	5.8-6.8	1.83	1.73-2.00
	68	11.2	10.6-12.2	6.0	5.5-6.5	1.86	1.69-2.12
	70	11.3	10.6-12.2	5.8	5.3-6.5	1.94	1.75-2.15
	71	12.0	10.4-12.8	6.2	5.7-6.7	1.94	1.77-2.17
	73	11.8	10.4-12.6	6.3	5.9-6.9	1.88	1.71-1.97
	121	11.9	10.4-12.6	6.4	5.7-7.1	1.88	1.77-2.00
	151	11.4	11.0-12.2	6.1	5.7-6.5	1.85	1.74-2.00
III	16	13.1	12.0-15.0	6.5	5.9-7.1	2.02	1.88-2.22
	72	13.1	12.0-14.6	6.5	5.9-6.9	2.01	1.84-2.20
	74	12.9	12.2-14.2	6.2	5.7-6.9	2.09	1.84-2.33

missed out from the t test calculations since the original fruit-body, though 4-spored, was exceptional in producing spores of one mating type only (Section 4.4.3). The most probable suggestion is that the fruit-body is monokaryotic but it is difficult to explain why its spores should be larger than the spores of the normal, dikaryotic fruit-bodies.

The spore shape and colour was found to be uniform throughout. The spore shape is ellipsoid-amygdaliform and the spore colour under the microscope is dark purplish-brown opaque to sub-opaque. There is a conspicupus germ pore 1-2 μm in diameter and a small apiculus.

Of all the spore characters which were examined, size appeared to be more variable than shape or colour and mean length was the most useful expression of spore size.

8.4.3 Basidia and cystidia

The following characters were examined in 8 isolates.

1. Basidium length.
2. Basidium width.
3. The length of cystidia on gill faces (pleurocystidia).
4. The width of pleurocystidia.
5. The length of cystidia on the gill edges (cheilocystidia).
6. The width of cheilocystidia.
7. The number of cheilocystidia per 1000 μm of gill edge.

The basidia and cystidia were separated from the gill tissue of herbarium specimens using the method of van Waveren (1971a). A wedge shaped segment of the cap was cut from the margin to centre using a razor blade. The segment was put under a binocular lens and the gills were carefully separated from the cap flesh with the help of mounted needles. The spores were washed off the gill with 10% NH_4OH by tapping the gill with a needle and were then blotted with filter paper. The edge of the

gill tissue was teased into small pieces, covered with a cover slip, and further dispersed by gentle tapping. In this way the basidia and pleurocystidia were separated from the rest of the cells, and could be located under the microscope. They were examined under high power and drawn using a Camera Lucida. The number of lageniform cystidia along the gill edge was counted. The gill edge was measured and the number of cheilocystidia which were found was converted to density per 1000 µm of gill edge. The cover slip was then tapped to separate some of the cheilocystidia and these were examined and drawn. Each drawn cell was measured with a ruler and the measurements were converted to µm. The width of cystidia at the neck, a character sometimes cited, was not measured since it was difficult to decide where to take the measurement. All the basidium and cystidium measurements for the 8 isolates which were examined are given in Table 8.5. One average basidium cheilocystidium and pleurocystidium of each of these isolates is drawn in Fig.8.5.

Although only a small number of isolates were examined it is possible to conclude that the size and shape of basidia and cystidia is too variable to be of use in distinguishing between breeding groups in this complex. The density of cystidia along the gill edge has been considered by van Waveren to be a diagnostic character between P.gracilis and P.microrrhiza. In this study more cystidia were found in isolates of Breeding Group II than in any other isolates but different densities were also found on different parts of the same gill edge and also between fruit-bodies of different ages.

8.4.4 Mycelial characters

Two mycelial characters useful in distinguishing between breeding

Fig. 8.5 Average basidia, chelocystidia and pleurocystidia in some isolates of the P.gracilis complex. Isolate numbers are indicated by the Arabic numbers and the breeding groups by the Roman numerals.

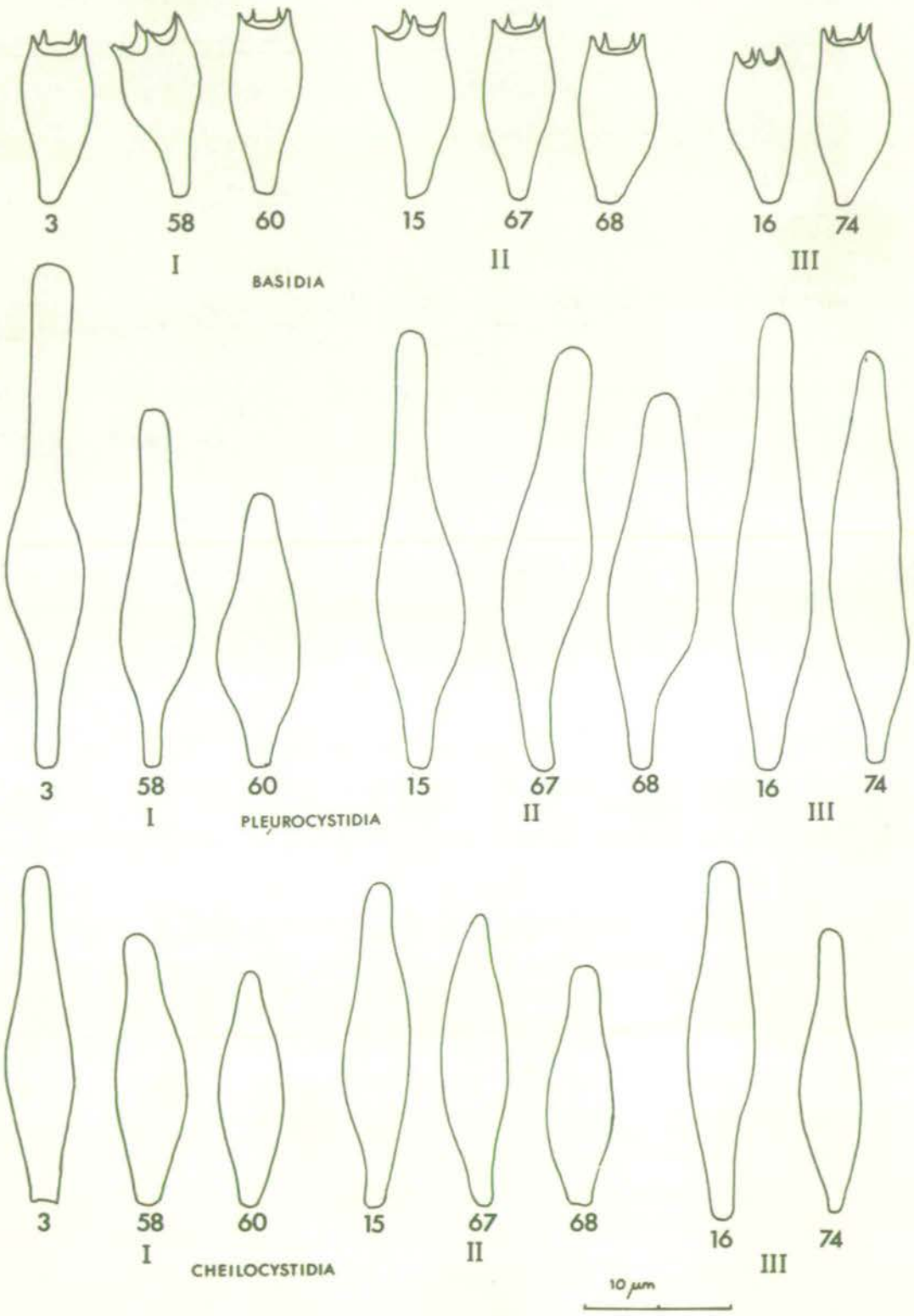

3
58
60
15
67
68
16
74
I
II
III
BASIDIA
3
58
60
15
67
68
16
74
I
II
III
PLEUROCYSTIDIA
3
58
60
15
67
68
16
74
I
II
III
CHEILOCYSTIDIA
10 µm

Table 8.5 Basidium and cystidium measurements in the P.gracilis group of isolates

Breeding Group	Isolate No.	Basidia				Pleurocystidia				Cheilocystidia				Number of cheilocystidia per 1000 µm of gill edge
		Length		Width		Length		Width		Length		Width		
		Mean (µm)	Range	Mean (µm)	Range	Mean (µm)	Range	Mean (µm)	Range	Mean (µm)	Range	Mean (µm)	Range	
I	3	24	21-26	11	10-13	64	57-73	11	10-15	41	32-49	10	7-12	30
	58	24	21-25	10	9-11	53	43-65	12	9-15	38	31-49	9	8-14	25
	60	21	19-25	11	10-12	39	26-46	12	11-13	36	30-35	11	8-13	24
II	15	23	20-29	10	9-13	63	54-71	11	10-12	36	25-56	8	6-12	45
	67	23	20-27	10	9-11	58	48-67	11	9-13	37	34-43	9	6-11	110
	68	20	17-22	10	8-11	45	34-54	11	10-14	33	29-36	10	9-13	70
III	16	23	20-25	11	10-11	71	52-81	10	8-13	43	38-48	9	8-10	32
	74	22	19-25	11	10-11	57	46-65	12	10-15	40	33-43	11	8-14	10

groups were noted.

1. Presence of 'rhizomorph strands'.

2. Colour of mycelial colonies.

'Rhizomorph strands' were formed on all mycelia derived from isolates of Breeding Group II. They were made of interwoven cords of hyphae and several of them were usually found radiating outwards from the centre of the mycelial colonies. They were formed on both malt and horse dung extract media, and on both monokaryotic and dikaryotic mycelia. Similar strands were sometimes produced in some of the isolates of Breeding Group III, but were never found in isolates of Breeding Group I.

The colour of monokaryotic mycelial colonies proved to be a useful character for distinguishing isolates of Breeding Group I. It was found that while most monokaryotic mycelia were white, a small but significant proportion was pink. After random basidiospore plating a few pink colonies were always found. The reasons for the colour difference were not investigated. No pink colonies were ever found in Breeding Groups II and III.

8.5 Discussion

The results of this chapter show that the British isolates of the P.gracilis complex can be divided into three genetically isolated breeding groups. The oidial homing responses reveal the relationship between them. A homing response followed by lethality is taken to be an indication of closer affinity than is a totally negative response and on this basis Breeding Groups I and III appear to be more closely related than either is to Breeding Group II. This conclusion is further corroborated by the aversion line evidence and the morphological comp-

arisons. Isolates belonging to Breeding Group II have a more robust fruit-body with a long rooting base, smaller basidiospores, and more frequent cheilocystidia. It is however difficult to make the distinction quickly and reliably on the basis of the morphological evidence alone.

In order to find out how the isolates would be identified on the basis of morphological evidence alone, the help of two morphological taxonomists was sought. Identical parcels were prepared, each containing the halved herbarium material of 6 isolates and a xeroxed copy of detailed morphological descriptions. One was sent to Dr. Kits van Waveren of the Leiden Herbarium, and the other to Prof. A. Smith of Michigan University. Two isolates of each of the three breeding groups were chosen, but the details of the breeding information were not forwarded. The results of the correspondence are summarised in Table 8.6.

Table 8.6 Identification of the same material by Dr. Kits van Waveren and Prof. A. Smith.

Isolate No.	Breeding Group	van Waveren	Smith
3	I	P.gracilis (Fr.)Quel.	P.gracilis (Fr.)Quel.
60	I	P.gracilis (Fr.)Quel.	near P.atomata (Fr.)Quel.
70	II	P.microrrhiza(Lasch.) Konr.&Maubl.	P.longicauda Karst. =P.microrrhiza sensu van Waveren
68	II	P.gracilis (Fr.)Quel.	P.orbitarum(Romagn.)Kuhner
16	III	P.gracilis (Fr.)Quel.	P.opacipes Smith
74	III	P.gracilis (Fr.)Quel.	near P.trepida(Fr.)Gillet

The table shows that quite different taxonomic conclusions were reached by these two eminent students of Psathyrella. Van Waveren's species concept is wide and with one exception (Isolate 68), his

results agree with the morphological conclusions reached in this chapter. The species concept of Smith is much narrower with a different name being given to each of the 6 isolates. Furthermore the 6 names fit into different parts of his classification. P.orbitarum belongs to the subsection Mesosporae whereas the others belong to the subsection Psathyrellae. The latter is further divided into the series Psathyrellae which includes P.gracilis, P.atomata, P.opacipes, and P.trepida; and the series Tenerae which includes the species P.longicauda. Obviously the isolates sent were not identical, but it is surprising that they should be considered different enough to merit their separation into 6 species. Smith's classification appears to be biologically meaningless.

Van Waveren's P.gracilis with the formae gracilis, corrugis, clavigera, albolimbata and substerilis is probably roughly equivalent to Breeding Groups I and III, although there is no direct information on the relationship of the formae to the breeding groups. His P.microrrhiza is probably equivalent to Breeding Group II.

The results presented in this chapter show that breeding information can be used in the classification of a set of closely related isolates. It is suggested that where no breeding information is available a wide morphological species concept should be adopted.

CHAPTER 9 : STUDIES ON THE P.CANDOLLEANA GROUP OF ISOLATES

9.1 Introduction

P.candolleana was described by Romagnesi as a collective species consisting of many varieties (Kuhner and Romagnesi, 1953). Breeding studies were therefore undertaken on a number of isolates belonging to this group. Independent studies were meanwhile being carried out by Mme. Galland under the supervision of M.H. Romagnesi. Her studies are now complete and are presented in a Ph.D. thesis (Galland, 1973). She crossed 29 isolates from France in all combinations and found four intersterile groups which were not clearly distinguishable on the basis of morphology.

In this study 27 different isolates were sampled from England, Finland and the U.S.A. Mycelia were crossed in order to establish breeding groups and homing responses were studied in order to investigate further levels of relationship. Unfortunately a request made to Galland for an exchange of stocks was not answered, so it was not possible to compare the identity of the groups established in this study with those found by Galland.

9.2 Material and methods

The 27 isolates used in this study are listed in Table 9.1. None was collected by myself since P.candolleana is not common in Scotland. Spore prints only were sent by Prof. A. Smith and these isolates are asterisked in Table 9.1. Exact details of the places of collection were often not given. Monokaryotic mycelia were established as described in Section 2.4, without heat treating the basidiospores. 90-100% basidiospore germination was observed in fresh isolates but the ability to germinate declined rapidly with age. Mycelia were crossed as described in Section 8.2. Homing responses were studied as described in Section 7.2.

Table 9.1 List of isolates belonging to the collective species P.candolleana

Cult.No.	Place of collection	Year of collection	Collector and No.
24	Cheboygan County, Michigan, U.S.A.	1969	R.W.6609
42	Yorkshire, England	1970	R.W.7560
43	Surrey, England	1968	P.O.3399
136	Warwickshire, England	1971	S.P. 575
141	Herefordshire, England	1970	M.R.
142	Turku, Finland	1971	R.W.8375
145	Turku, Finland	1971	R.W.8412
146	Helsinki, Finland	1971	R.W.8561
160	Michigan, U.S.A.	1972	A.S.81096*
161	Michigan, U.S.A.	1972	A.S.81097*
162	Michigan, U.S.A.	1972	A.S.81098*
163	Michigan, U.S.A.	1972	A.S.81099*
165	Michigan, U.S.A.	1972	A.S.81103*
166	Michigan, U.S.A.	1972	A.S.8112*
167	Bellville, Michigan, U.S.A.	1972	F.H.1974*
168	Michigan, U.S.A.	1972	A.S.81113*
169	Michigan, U.S.A.	1972	R.N.204*
170	Michigan, U.S.A.	1972	R.N.208*
171	Kent, Ohio, U.S.A.	1972	F.H.2096*
173	Greenville, Michigan, U.S.A.	1972	A.S.-*
175	Michigan, U.S.A.	1972	A.S.81153*
176	Michigan, U.S.A.	1972	A.S.81156*
178	Michigan, U.S.A.	1972	F.H.2217*
179	Michigan, U.S.A.	1972	F.H.2194*
300	- **	1947	Pinto Lopes (C.B.S.
304	- **	1933	Vandendries (C.B.S.)
320	- **	1939	Quintanilha (C.B.S.)

* Isolates sent in by Prof. A. smith in the form of spore print only.
** Place of collection not known.

Abbreviations:
C.B.S. - Centraalbureau voor Schimmelcultures, Baarn; F.H. - Florence Hoseney; R.N. - Rosemary Nimke; P.O. - Peter Orton; S.P.- Stanley Porter; M.R. - Maurice Rothero; A.S. - Alexander Smith; R.W. Roy Watling.

9.3 Results

Monokaryotic mycelia of the 27 isolates were crossed in most combinations and four breeding groups were found (Table 9.2). The results of crossing 12 selected isolates are shown in Fig. 9.1. Isolates 141 and 142 were then chosen as representative of Breeding Groups I and II respectively and mycelial crossings between the four mating types of these representative isolates and all the remaining isolates were made. The results are summarised in Fig.9.2. Isolate 141 from Herefordshire, England and Isolate 179 from Michigan, U.S.A. had one mating type in common. A common mating type was also found between Isolates 42 from Yorkshire, and Isolate 43 from Surrey. Different mating types were found in all the other pairs of isolates. No obvious aversion lines were found in any of the mycelial crossings.

Table 9.2 Breeding groups and countries of origin in the P.candolleana group of isolates. (C.B.S. = Centraalbureau voor Schimmelcultures, Baarn; place of collection not known).

Breeding Group	Isolate Nos.	Country of Origin
I	24, 160, 161, 162, 163, 165, 166, 167, 168, 169, 170, 173, 175, 176, 179.	U.S.A.
	141	England
	145	Finland
II	42, 43, 136	England
	146	Finland
	171	U.S.A.
	304, 320	(C.B.S.)
III	178	U.S.A.
IV	300	(C.B.S.)

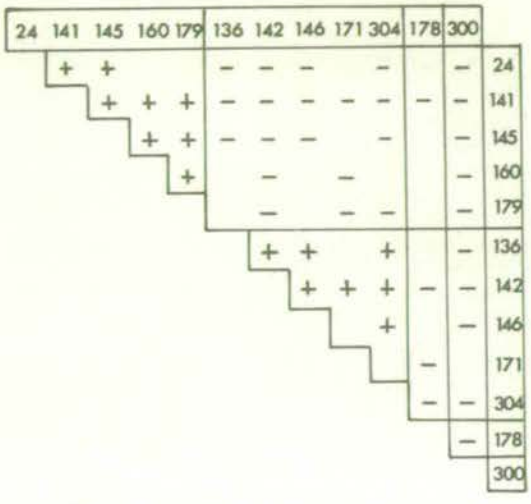


Fig. 9.1 Results of mycelial crossing between the P.candolleana group of isolates.

+ dikaryon with clamps formed
- dikaryon with clamps not formed

	24	42	43	136	145	146	160	161	162	163	165	166	167	168	169	170	171	173	175	176	178	179	300	304	320
141	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-
142	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+

Fig. 9.2 Results of mycelial crossing between Isolates 141 and 142 and all the remaining P.candolleana isolates

+ dikaryon with clamps formed
- dikaryon with clamps not formed

Homing responses were studied between 12 selected isolates. The results are shown in Fig. 9.3. Reciprocal lethal reactions were observed between most pairs of isolates of breeding Groups I and II and between II and III. Some examples have been photographed and are shown in Figs. 7.7-7.10. Isolate 300 never produced any oidia and so reciprocal responses could not be studied.

A few of the microscopic and cultural characters were compared within and between the breeding groups. Macroscopic characters of the fruit-body were not considered since almost all the American isolates were available in the form of spore print only. Isolate 300 was available in the form of mycelium only.

Basidiospore shape, size and colour were compared in Isolates 24, 141, 145, 42, 136, 142, 146 and 178. In each of these isolates at least 20 spores were drawn using a Camera Lucida. Their mean length and width measurements are given in Table 9.3. A few spores of each isolate are drawn in Fig.9.4. Although some differences in spore size and shape were found these are not correlated with the breeding groups. Spore colour appeared to be more or less uniform. Basidiospores of all isolates germinated without a noticeable vesicle in the germ tube. Mycelia belonging to all isolates, except Isolate 300, released a yellow pigment into the culture medium. Isolate 300 had a distinctly more aerial mycelium, but this character could have been acquired by mutation during the thirty years of repeated subculturing in the culture collection. Mating patterns were investigated only in Isolates 141 and 142 and in both the bifactorial incompatibility system was found.

Only once was a P.candolleana fruit-body produced in culture

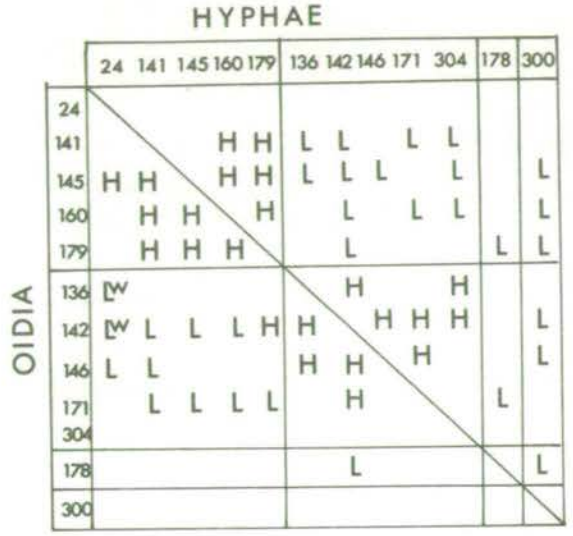


Fig. 9.3 Homing responses between selected isolates in the P.candolleana group of isolates.

H Homing of hyphae towards oidia.

L Homing of hyphae towards oidia followed by a lethal reaction.

L^W Homing of hyphae towards oidia followed by a weak lethal reaction.

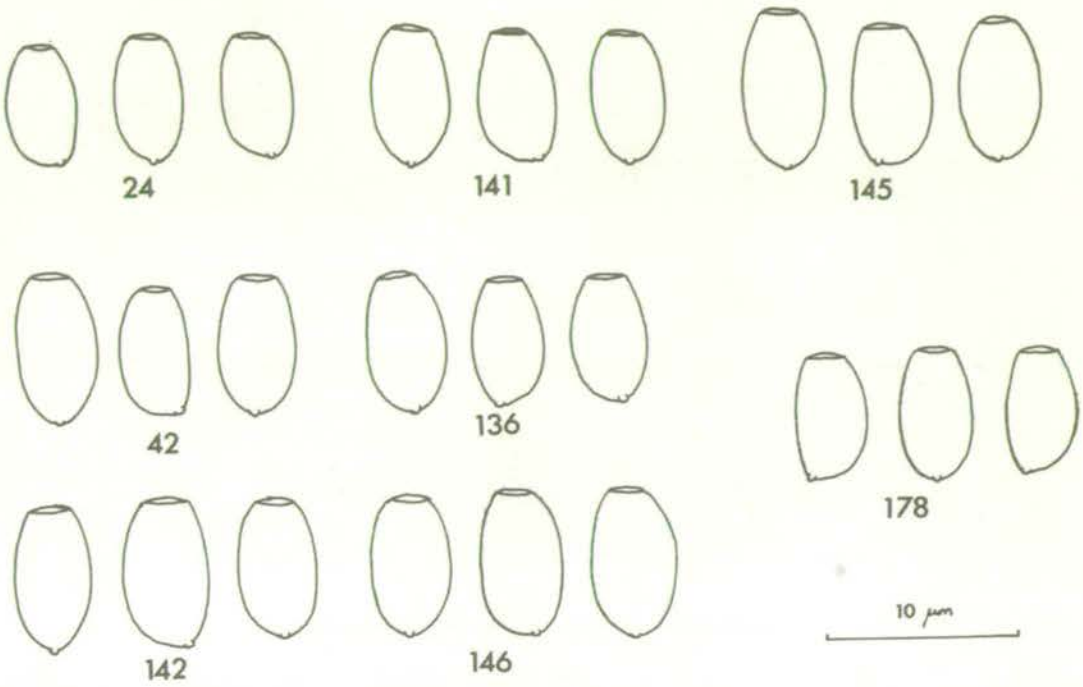


Fig. 9.4 Average basidiospores in the P.candolleana complex. Isolate numbers are indicated.

Table 9.3 Basidiospore measurements in the P.candolleana complex.

	Isolate No.	Length		Width		Length/Width Ratio	
		Mean (µm)	Range	Mean (µm)	Range	Mean	Range
I	24	6.9	6.1-7.7	3.9	3.5-4.3	1.79	1.58-2.00
	141	7.3	6.9-7.7	4.3	4.1-4.7	1.70	1.55-1.85
	145	7.7	6.8-8.5	4.3	3.8-4.7	1.78	1.58-2.00
II	42	7.4	6.9-8.1	4.1	3.7-4.5	1.83	1.68-2.00
	136	7.1	6.5-7.7	4.3	3.9-4.9	1.68	1.50-2.00
	142	8.0	7.5-9.1	4.4	4.1-4.9	1.78	1.58-2.00
	146	7.6	7.0-8.3	4.2	3.8-4.5	1.80	1.58-2.05
III	178	6.8	6.2-7.5	4.0	3.8-4.7	1.70	1.50-1.85

on a mycelial crossing plate between Isolate 142 (Finland) and Isolate 171 (U.S.A.). Perhaps the dikaryon derived from a combination of two geographically separated and therefore genetically different monokaryons was a particularly vigorous one. A photograph of this fruit-body is shown in Fig.9.5.

9.4 Discussion

The results of mycelial crossing between English, Finnish and American isolates of the P.candolleana complex are similar to the results of Galland (1973) who used French isolates only. Four breeding groups were found in both studies. The results of this study show that even though the breeding groups cannot exchange genetic information, homing and fusion still take place. The block to genetic exchange occurs after cell fusion, due to an antagonistic reaction between the two different nuclei. The occurrence of homing and fusion between the hyphae and oidia of each of the breeding group combinations suggests that the four breeding groups are closely related. However, the tests give no further information on the relative affinity of the breeding groups to one another.

The geographical distribution of the four groups is interesting since it shows that the same breeding group can be found in different parts of the world and also that different breeding groups can be found in the same area. Breeding Groups I and II were each found in England, Finland and the U.S.A. Since the divergence between the groups is unlikely to have occurred more than once in different parts of the world, the basidiospores must somehow have travelled across the Atlantic. It is possible for spores as small as these to be air dispersed in spore masses, and it is also likely that some spores were imported with

Fig. 9.5 Fruit-body of P.candolleana Breeding Group II produced in culture on a mycelial crossing plate between Isolates 142 and 171.

tree seedlings.

In Finland Isolates 142 (Breeding Group II) and 145 (Breeding Group I) were found growing in the same habitat and were collected on the same day. Clearly, it is not geographical factors nor, in all probability, ecological factors that maintain the isolation of these two groups. The fundamental biological distinction between the groups is indicated by the lethal reaction, and this may not be reflected by any morphological differences.

The taxonomic status of the groups needs further investigation and no decisions can be made without a more thorough morphological study. Galland (1973) carried out some morphological studies on her material and found several small differences in spore colour and cystidium shape. However, there was no correlation between these differences and the breeding evidance. In spite of this Romagnesi decided to give a different specific name to each of the breeding groups and suggested the ad interim names P.candolleana, P.elegans, P.proxima and P.scotospora. If the differences between the groups are indeed as slight as they suggest the usefulness of inventing three new species names may be in question. Romagnesi's original suggestion of a collective morphospecies (Kuhner and Romagnesi, 1953) would seem to be more sensible.

CHAPTER 10 : STUDIES ON THE COPROPHILOUS ISOLATES

10.1 Introduction

Since only a few coprophilous Psathyrella species have been described in Britain, all the available isolates were included in the study. The isolates could be divided into two complexes on the basis of fruit-body morphology. Complex 1 isolates were identified by a dark brown cap and a conspicuous flocculate veil on the cap and stipe. Complex 2 isolates were identified by a dark red cap, no macroscopically distinct veil and a smooth stipe. However, there was morphological variation within each complex and it was difficult to decide on the limits of the species. Breeding studies were undertaken in an attempt to sort out the taxonomy.

Nine Complex 1 isolates and seven Complex 2 isolates were used in this study. Some of the isolates were collected by myself. Most were obtained from the Herbarium of the Royal Botanic Garden, Edinburgh. The isolates belonging to Complex 1 were always labelled P.coprobia by other collectors while the isolates belonging to Complex 2 were usually left unnamed. The Complex 2 isolates were considered by Dr. R. Watling (Royal Botanic Garden, Edinburgh) to be possible material for the description of one or more new species, and so when 2 breeding groups were discovered the material was passed on to him for a morphological examination. Using morphological and breeding evidence, Watling decided to establish two new species which he called P.coprophila and P.fimetaria. These findings were soon published under joint authorship (Watling and Jurand, 1971). The part on the morphological comparisons was written by Dr. Watling and the part on the culture studies by myself. Since then further studies on the group

have been carried out by Dr. Kits van Waveren in Holland. After extensive morphological studies on other isolates and also on the borrowed type material, van Waveren decided that the morphological differences between P.coprophila and P.fimentaria were insufficient to warrant the description of two new species. Ignoring the evidence of the sterility barrier he decided to reunite the two species under the name P.coprophila (van Waveren, 1972). Since the publication of the paper (Watling and Jurand, 1971) an extensive correspondence has been exchanged with Dr. van Waveren, more breeding information has become available, and a clearer impression of the taxonomy of the group is therefore now possible.

10.2 Materials and methods.

The isolates used in this study are listed in Table 10.1. Cow, bull or horse dung was the substrate on which isolates were usually found, but since the dung was several months old at the time of colonisation, it was difficult to decide on its exact origin. The isolates were placed in one of the two complexes on the basis of gross morphology. The two complexes are referred to as the P.coprobia and the P.coprophila complexes respectively.

Monokaryotic mycelia were established as described in Section 2.4 without heat treating the basidiospores. Good germination was observed in fresh isolates and, although the percentage germination declined gradually with age, even some five year old isolates had a few viable spores.

Mycelial crossing and homing tests were carried out separately in the two complexes and some inter-complex tests were also made.

Table 10.1 List of coprophilous isolates.

Isolate No.	Place of collection	Map grid reference	Month and year of collection	Collector and No.
P.coprobia complex				
14*	Midlothian, Scotland		Sept. 1966	R.W.5243
30	Mull, Scotland		Sept. 1968	R.W.7814
31	Mull, Scotland		Sept. 1968	R.W.7843
32	Hilversum, Holland		- 1970	J.D.
33	Perthshire, Scotland	NO 571 091	Aug. 1970	M.J.
40*	Mull, Scotland		Sept. 1968	R.W.7756
87	Devon, England	SK 701 764	Sept. 1971	M.J.
88	Devon, England	SX 613 775	Sept. 1971	M.J.
133	Perthshire, Scotland		Sept. 1972	A.B.
P.coprophila complex				
1*	Midlothian, Scotland		Nov. 1966	R.K.(R.W.3947)
51*	Perthshire, Scotland		Aug. 1967	R.W.7440
59	Perthshire, Scotland	NO 635 056	Aug. 1970	M.J.(R.W.7470)
84*	Midlothian, Scotland	NT 185 590	Aug. 1971	M.J.
128*	Uppsala, Sweden		Aug. 1971	R.W.
129*	Uppsala, Sweden		Aug. 1971	R.W.
144*	Perthshire, Scotland		Aug. 1967	R.W.5069

Abbreviations:

A.B. - Angela Ballantyne; J.D. - J. Daams; M.J. - myself; R.K. - Roger Kemp; R.W. - Roy Watling.

*Isolates which produced fruit-bodies in culture.

Mycelia were crossed as described in Section 8.2. Homing responses were studied as described in Section 7.2. Attempts to produce fruit-bodies in culture were made and these are described in Section 10.4 of this chapter.

10.3 Results of mycelial crossing and homing tests

The results of mycelial crossing tests between the 9 isolates of the P.coprobia complex (Fig.10.1) show that the complex consists of three breeding groups. The results of homing tests (Fig.10.2) show positive homing responses in tests involving isolates of the same breeding group and no response at all in tests involving isolates of different breeding groups. Lethal reactions were never observed.

The results of mycelial crossing between the 7 isolates of the P.coprophila complex (Fig.10.3) show that the complex consists of two breeding groups. No homing responses were observed between Breeding Groups I and II (Fig.10.4).

Negative results were also obtained in all mycelial crossing and homing tests involving different complexes (Figs. 10.5 and 10.6). No obvious aversion lines were noticed in any of the mycelial crossings.

10.4 Fruit-body production in culture

All isolates belonging to Breeding Group I of the P.coprophila complex produced fruit-bodies in culture. Fruiting was not very successful in the other breeding groups and several different conditions were tried in order to improve fruit-body production. The following media were used:

1. Horse dung extract agar
2. Malt agar.
3. Sterile horse dung.

Fig. 10.1

	30	31	32	33	40	87	88	133	14
30		+	−	−	−	−	−	−	−
31			−	−	−	−	−	−	−
32				+	+	+		+	−
33					+	+			−
40						+	+	+	−
87							+	+	−
88								+	−
133									−
14									

Fig.10.2 HYPHAE

OIDIA	30	31	32	33	40	87	88	133	14
30		H		−	−				−
31	H			−	−				−
32					H				
33	−	−	H			H			−
40							H	H	
87								H	H
88							H		−
133							H		
14	−	−		−	−				

Fig. 10.3

	1	51	84	128	129	144	59
1		+	+	+	+	+	−
51			+	+	+	+	−
84				+	+	+	−
128					+	+	−
129						+	−
144							−
59							

Fig.10.4 HYPHAE

OIDIA	1	51	84	128	129	144	59
1		H	H	H	H		−
51	H		H	H	H		−
84	H	H					−
128	H	H					−
129	H	H		H			−
144							−
59	−	−	−	−	−		

Fig.10.5

		I	II
		1	59
I	30	−	−
II	33	−	−
III	14	−	−

Fig.10.6 HYPHAE

OIDIA		I	II	III	I	II
		30	33	14	1	59
I	30		−	−		
II	33	−		−	−	
III	14		−		−	
I	1			−		−
II	59		−			

\- Dikaryon with clamps formed
H Homing of hyphae towards oidia
\- Dikaryon with clamps not formed
or no homing of hyphae towards oidia

Fig. 10.1 Results of mycelial crossing in the P.coprobia complex.

Fig. 10.2 Homing responses in the P.coprobia complex.

Fig. 10.3 Results of mycelial crossing in the P.coprophila complex.

Fig. 10.4 Homing responses in the P.coprophila complex.

Fig. 10.5 Results of mycelial crossing between isolates of the P.coprobia and P.coprophila complexes.

Fig. 10.6 Homing responses between isolates of the P.coprobia and P.coprophila complexes.

4. Sterile horse dung over a layer of dung extract agar.
5. Sterile horse dung and straw over a layer of dung extract agar.
6. Sterile soil over a layer of dung extract agar.
7. Sterile soil and straw over a layer of dung extract agar.

Three types of containers were also tried.

1. Petri dishes.
2. Wide necked glass milk bottles plugged with cotton wool.
3. Small jars with loosely fitting metal lids.

By the end of the fruiting experiment adequate fruit-bodies were produced in three of the five breeding groups. All the isolates which produced fruit-bodies in culture are indicated in Table 10.1. Isolates of P.coprophila Breeding Group I produced fruit-bodies on all media containing dung or dung extract. Isolates 33 and 40 of P.coprobia Breeding Group II fruited best on sterile dung in a glass jar. Isolate 14 of P.coprobia Breeding Group III sometimes produced adequate fruit bodies on dung extract agar in petri dishes and the frequency of fruiting was not improved by any of the other media or containers used. Isolates of the other two breeding groups never produced any fruit-bodies in culture.

In isolates of P.coprophila Breeding Group I a connection was noticed between the method of inoculation and the speed of fruit-body production. When a mixture of compatible monokaryons was inoculated on a plate fruit-bodies were formed more rapidly than when a dikaryotic mycelium only was inoculated. A simple experiment was carried out to investigate this further.

In each plate 9 inoculum plugs were placed in the form of a 3 x 3 square. The first set of plates was inoculated with 9 identical

dikaryotic plugs. In the second set compatible inoculum plugs were placed alternately in a 3 x 3 square. In the third set 9 identical monokaryotic plugs were inoculated. In each set 7 plates were used, each inoculated with a different culture. The 21 plates were incubated in the light at room temperature and were examined daily for the formation of fruit-bodies. The number of days since inoculation when the first mature fruit body was found was noted for each plate, and the results are given in Table 10.2.

Table 10.2 Number of days since inoculation when the first mature fruit-body was found. The plates were inoculated with 1) dikaryotic inocula only 2) compatible monokaryotic inocula 3) monokaryotic inocula only. All cultures used are of P.coprophila Breeding Group I.

1.) Cult.No.of dikaryotic inocula	Days	2.) Cult.No.of compatible monokaryotic inocula	Days	3.) Cult.No.of monokaryotic inocula	Days
51.(1x2)	-	1.1x1.7	14	1.7	-
51.(1x4)	20	1.1x51.1	13	51.1	-
51.(2x3)	-	1.1x84.4	12	51.3	-
84.(1x2)	14	1.1x144.1	14	84.2	23
84.(2x3)	22	51.1x51.3	12	128.3	-
129.(4x5)	19	84.1x84.4	11	129.4	-
129.(6x4)	-	129.1x129.5	13	144.1	26

The results show that fruiting occurs most readily in plates inoculated with a mixture of compatible monokaryons. It may be that the physical act of hyphal fusion stimulates the formation of fruit-body primordia. Fruit-bodies formed on plates inoculated with plugs of one monokaryotic culture only (Table 10.2, part 3) are made of monokaryotic hyphae and their morphology is discussed later.

10.5 Observation on fresh fruit-bodies

In Figs. 10.7-10.9 are shown normal dikaryotic fruit-bodies of Isolate 84 (P.coprophila Breeding Group I). The fruit-body on Fig.10.7 was photographed soon after collection. The fruit-bodies on Figs. 10.8 and 10.9 were grown in culture. The differences in gross morphology are most likely due to the different culture environments. In Figs. 10.10 and 10.11 are shown normal dikaryotic fruit-bodies of Isolate 33. The fruit-body on Fig.10.10 was photographed in the field before collection. The fruit-body on Fig.10.11 was grown in culture. There is no difference in gross morphology between the naturally growing and cultured fruit-bodies. A cultured fruit-body of Isolate 14, P.coprobia Breeding Group III is shown on Fig.10.12. This fruit-body does not resemble the fruit-bodies of either P.coprophila Breeding Group I (Figs.10.7-10.9) or P.coprobia Breeding Group II (Figs.10.10 and 10.11).

Figs. 10.7-10.12 show normal dikaryotic fruit-bodies of three different coprophilous breeding groups. In Figs. 10.13-10.16 are shown some abnormal fruit-bodies of P.coprophila Breeding Group I. Monokaryotic fruit-bodies were found to be the most common abnormality in culture (Fig.10.13). The differences between the monokaryotic and dikaryotic fruit-bodies are summarised on Table 10.3. Spore density is the most important difference since it is responsible for the differences in colour. Monokaryotic fruit-bodies produced only a few spores all of the original mating type. One difference which is independent of spore density is stipe posture. The stipes of dikaryotic fruit-bodies were firm and erect while those of monokaryotic fruit-bodies lacked directed growth. This is thought to be due to a difference in their response to gravity.

Fig. 10.7 Isolate 84 (P.coprophila Breeding Group I) photographed soon after collection.

Fig. 10.8 Cultured dikaryotic fruit-body of Isolate 84 grown on horse dung extract in a petri dish. The lid of the petri dish had been lifted before the stipes elongated and the dish was covered with an upturned beaker.

Fig. 10.9 Cultured dikaryotic fruit-body of Isolate 84 (P.coprophila Breeding Group I) grown in a wide necked milk bottle in dung and straw over a layer of horse dung extract agar.

Fig.10.10 Isolate 33 (P.coprobia Breeding Group II) photographed in the field.

Fig.10.11 Cultured dikaryotic fruit-body of Isolate 33 (P.coprobia Breeding Group II) grown on sterile dung in a small jar.

Fig. 10.12 Cultured dikaryotic fruit-body of Isolate 14 (P.coprobia Breeding Group III) grown on horse dung extract agar in a petri dish.

Fig.10.13 Cultured monokaryotic fruit-body of Isolate 84 (P.coprophila Breeding Group I) grown on horse dung extract agar.

Fig.10.14 Abnormal fruit-body of Isolate 1 (P.coprophila Breeding Group 1) shown in culture. The fruit body has gills but no cap.

Fig.10.15 Abnormal fruit-body of Isolate I (P.coprophila Breeding Group I) grown in culture. The fruit-body has an extra set of gills crowning the cap.

Fig.10.16 This mass of tissue has no recognisable fruit-body structure and no basidiospores, but its gills are covered with numerous utriform cystidia which are indistinguishable from the cystidia of normal, dikaryotic fruit-bodies.

Table 10.3 A comparison between the dikaryotic and monokaryotic fruit-bodies of P.coprophila Breeding Group I.

	Dikaryotic fruit body	Monokaryotic fruit-body
colour of cap	snuff-brown	fulvous
size of cap	5 - 15 mm	5 - 11 mm
length of stipe	20- 50 mm	10- 35 mm
posture of stipe	erect	almost prostrate
colour of gill	cigar-brown	fawn
colour of gill edge	cigar-brown	fawn
spore density	abundant	few
colour of spore print	fuscous-black	-
spore size	12.5 - 14.0 x 6.5 µm	12.5 - 14.0 x 6.5 µm
spore number per basidium	4	4
cystidium shape	utriform	utriform
cystidium frequency	absent or infrequent	abundant

Three other abnormal fruit-bodies were formed spontaneously in dikaryotic cultures on agar plates. Each was observed only once. The fruit-body shown in Fig.10.14 had gills but no cap while those shown in Fig.10.15 had an extra set of gills crowning the cap. The mass of tissue shown in Fig.10.16 had no recognisable fruit-body structure and no basidiospores but the folds of the tissue were covered with numerous utriform cystidia which were indistinguishable from those found on the gills of normal fruit-bodies.

It is possible that the artificial environment of the culture plates might encourage the formation of abnormal forms. If found in nature, such abnormal fruit-bodies may baffle the collector and may consequently come to be wrongly classified in different genera or even classes of fungi.

10.6 Taxonomy of the P.coprobia complex

The complex consisted of three breeding groups as follows:

I : Isolates 30, 31.

II : Isolates 32, 33, 40, 87, 88, 133.

III : Isolate 14.

Only isolates of Breeding Group II have been seen growing in their natural habitat. All appeared to be similar in gross morphology. The cultured fruit-bodies of Isolate 14 (Breeding Group III) differed in gross morphology from all the naturally grown and cultured specimens of Breeding Group II. The cap was generally darker and more furrowed, the veil less conspicuous, and the stipe darker and smoother in texture. The microscopic differences were not so pronounced. The cheilo- and pleurocystidia were lageniform like those of other P.coprobia isolates. The basidiospores appeared to differ slightly in shape, being broader and more angled at the apicular end (Fig.10.17 and Table 10.4). The only significantly different culture character was to be found in the incompatibility system. The mating system of Isolate 14 has been found to be determined by three factors whereas the mating system of the other P.coprobia isolates was clearly bifactorial.

Fresh fruit-bodies of the two isolates belonging to Breeding Group I were not studied. Only basidiospore characters were examined. The spores of Isolates 30 and 31 appeared to be slightly narrower than those of the other isolates but there was no difference in length (Fig. 10.17, Table 10.4). The measurements were not compared statistically.

It is possible that in the future these three intersterile breeding groups will each be given specific status. However the decision to describe new species must be delayed until more isolates belonging to Breeding Groups I and III are found.

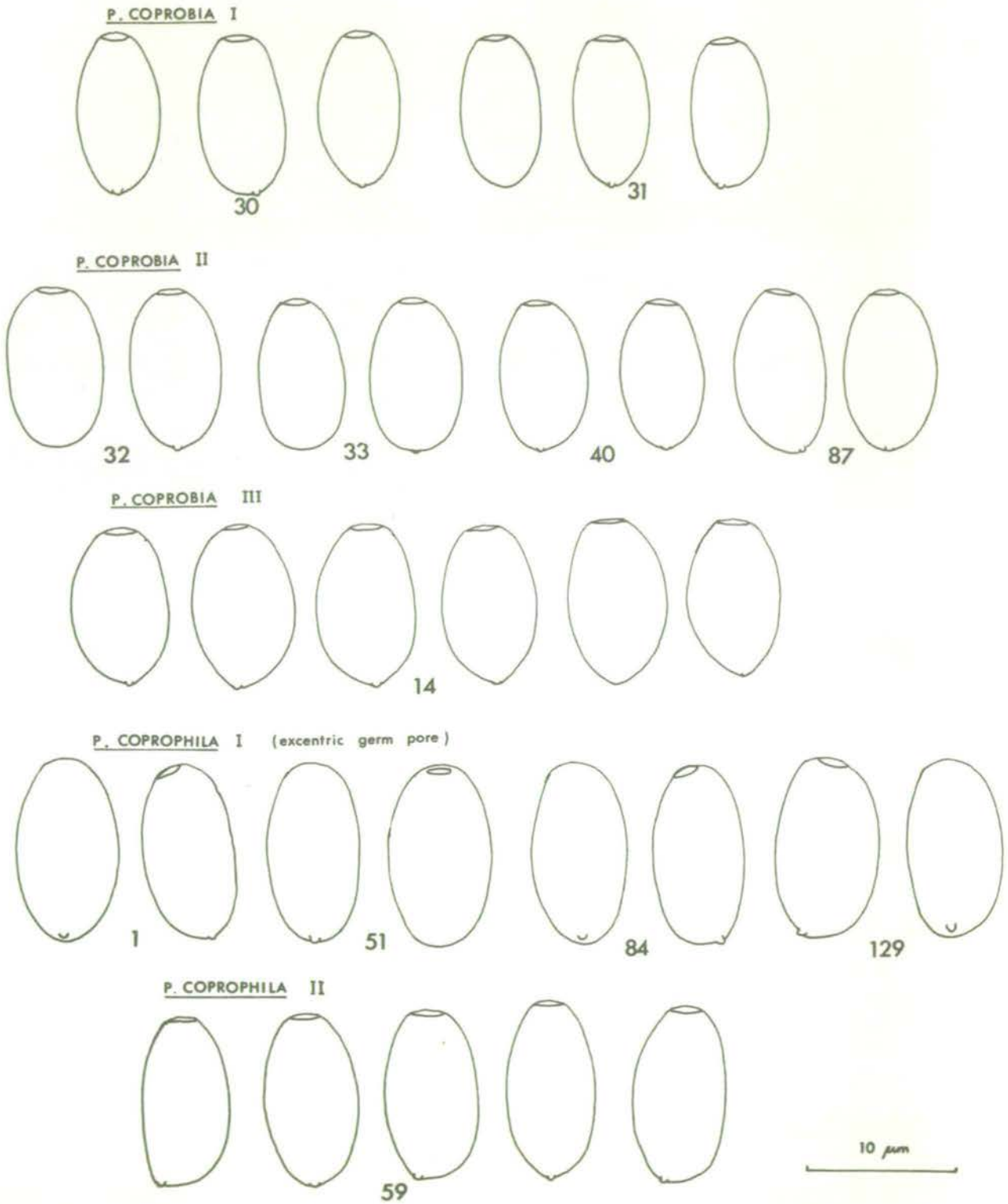


Fig. 10.17 Average basidiospores of some coprophilous isolates. Isolate numbers are indicated.

Table 10.4 Basidiospore measurements of the coprophilous isolates

Group	Isolate No.	Length		Width		Length/Width Ratio	
		Mean (µm)	Range	Mean (µm)	Range	Mean	Range
P.coprobia complex							
I	30	11.2	10.6-11.8	5.9	5.6-6.3	1.89	1.77-2.07
	31	10.7	9.8-11.8	5.7	5.3-6.5	1.86	1.66-2.04
II	32	11.1	10.6-11.8	6.1	5.7-6.7	1.82	1.69-2000
	33	10.4	9.8-11.0	6.1	5.5-6.5	1.70	1.55-1.85
	40	10.3	9.8-10.6	6.0	5.5-6.5	1.72	1.61-1.85
	87	11.5	10.6-12.4	6.4	5.7-7.1	1.79	1.66-1.93
	88	11.3	10.6-12.4	6.4	5.7-6.7	1.77	1.68-1.89
III	14	11.2	10.6-12.0	6.7	6.2-7.2	1.68	1.54-1.81
P.coprophila complex							
I	1	12.3	11.4-13.4	6.4	5.7-7.1	1.92	1.75-2.10
	51	12.6	11.4-13.4	6.6	6.1-6.9	1.89	1.72-1.99
	84	13.3	12.2-14.6	7.2	6.3-7.7	1.84	1.68-2.00
	129	12.8	12.0-13.4	6.9	6.5-7.5	1.85	1.71-2.00
II	59	12.3	12.0-12.8	6.3	6.1-6.5	1.85	1.80-1.98

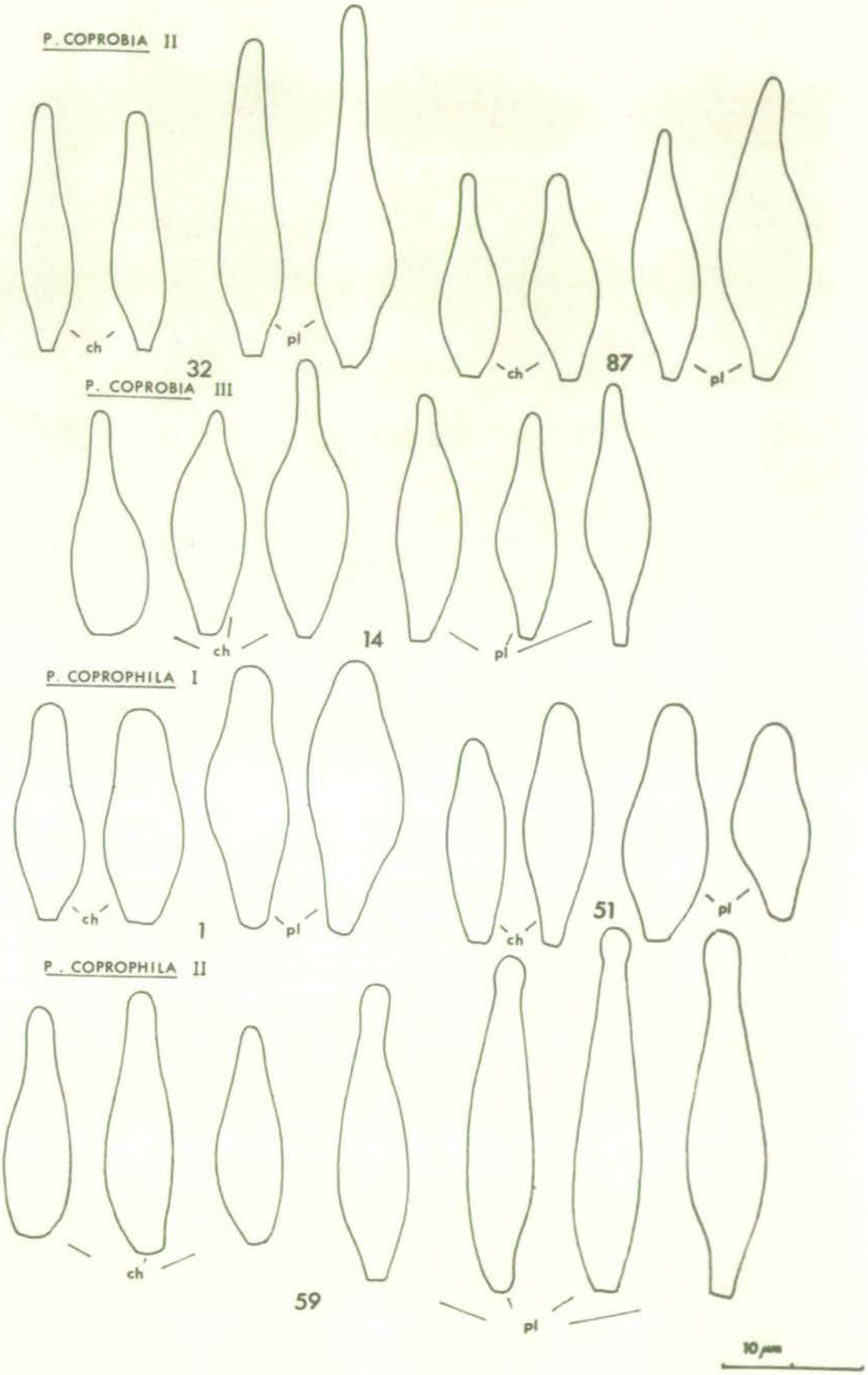


Fig. 10.18 Average cheilocystidia (ch) and pleurocystidia (pl) of some coprophilous isolates. Isolate numbers are indicated.

10.7 Taxonomy of the P.coprophila complex

The complex includes two breeding groups as follows.

I : Isolates 1, 51, 84, 128, 129, 144

II : Isolate 59

Isolate 59 differs from all the other isolates in this complex in having a central, not excentric germ pore (Fig. 10.17) and lageniform, not utriform cystidia (Fig. 10.18). In these characters it resembles the isolates of the P.coprobia complex. It was, however, placed in the P.coprophila complex on account of its large basidiospores, reddish brown cap and no macroscopically visible veil.

The taxonomic treatment proposed in the preliminary account (Watling and Jurand, 1971) is very misleading since the morphological investigations were not properly coordinated with the breeding evidence. At the time of the publication only Isolates 1 and 59 were available for culturing. Since sterility had been found between them Watling decided that two species were involved. When describing the new species he used the original fruit-body of Isolate 1 as the type of P.coprophila and matched Isolate 59 with the holotype of P.fimetaria. Unfortunately the fruit-body which he chose for the holotype of P.fimetaria was too old to culture and it is a pity that he did not use Isolate 59 as the holotype instead. This fact is responsible for considerable confusion, for although Isolate 59 may represent a truly different species, some of the isolates described under the name P.fimetaria are in fact P.coprophila. For example, Isolate 51 derived from a paratype of P.fimetaria (Accession Number R.W.7440), has since the publication of the paper been shown to cross with the type of P.coprophila.

Van Waveren, after a thorough study of the published descriptions and a morphological examination of some of the borrowed material came to the conclusion that P.coprophila and P.fimetaria are conspecific (van Waveren, 1972). Significantly, however, he agreed that Isolate 59 is different from all the other isolates and was the first to point out the differences in germ pore position and cystidium shape. In the morphological section of the paper (Watling and Jurand, 1971), he found the following errors and omissions.

1. In the description of P.coprophila the pleurocystidia are said to be "absent or very rare" while in the description of P.fimetaria the cystidia are said to be just "present". This difference is even made into a key character, but in the key it is the other way round.

2. The dimensions of cheilocystidia are given for P.coprophila but are missing from the descriptions of P.fimetaria.

3. The pleurocystidia of P.fimetaria are said to be "similar to the narrowest cheilocystidia or fusiform". The cheilocystidia are described as "elongate or narrowly lageniform". But in the figures, while the cystidia are indeed lageniform, the pleurocystidia are strikingly plump, the very opposite of narrow.

4. In the basidiospores of P.coprophila the germ pore is said to lie excentrically on the abaxial side, but the position of the germ pore in P.fimetaria is not mentioned.

To summarise, the taxonomic position of the complex is now as follows. Breeding Group I contains both the holotype of P.coprophila and the paratype of P.fimetaria. Moreover, van Waveren's morphological studies have shown no significant differences between the two holotypes.

The two 'species' must therefore be considered synonymous. Breeding Group II (Isolate 59) may represent a new species but it should not be given a formal name until the morphological differences between the two groups are confirmed in further material interfertile with Isolate 59.

The idea of joining the cultural and morphological information together in one paper is a good one but requires proper co-ordination. In this case I first saw the morphological section of the paper in proof form and was not aware until then that in the two sections of the paper different types of P.fimetaria had been used.

CHAPTER 11 : GENERAL DISCUSSION

Psathyrella is a difficult genus to classify consisting of large numbers of apparently closely related taxa. It has been the object of this study to investigate the reasons for this taxonomic complexity, to delimit breeding groups, to suggest what leads to their formation and as far as possible to arrive at a practical and at the same time biologically meaningful species concept.

The reasons for the lack of clear discontinuities are to be found in the flexibility of the breeding system. Each cell of the mycelium is capable of mutation and propagation. It has even been suggested that mutation may be the main source of variability in the fungi (Snyder, 1958). The haploid condition of the monokaryotic mycelium allows a mutant to be immediately exposed to selection. Each cell of the mycelium is capable of fusion. Fusion can take place at any time between monokaryotic hyphae, between monokaryotic and dikaryotic hyphae, and between hyphae and oidia. Thus two adjacent mycelia of the same breeding group do not remain separate for long, but soon become part of an enormous network of hyphae. This ability of hyphal cells to fuse without the requirement of complicated sexual apparatus is the fundamental difference between the Basidiomycetes and the Angiosperms. In the Angiosperms an adult plant is an individual and its genotype is fixed for life. In the Basidiomycetes there are no discrete individuals, only inter-connected mycelia each a potential mosaic of several genetically different individuals. Genetical mosaics can be synthesized easily on agar plates. Evidence for their occurrence in nature has been given by Saunders (1956) for *Polyporus betulinus* and by Burnett and Partington (1957) for *Polystictus versicolor*.

Fusions of hyphal cells, or of hyphal cells and oidia result

in dikaryons if the fused cells are compatible. The Psathyrella isolates examined in this study have been shown to have the bifactorial incompatibility system with the exception of one which is thought to have a trifactorial system. This means that interbreeding in the form of sister-mating is reduced to 25% in the case of a bifactorial system and 12½% in the trifactorial system. The promotion of outbreeding by the incompatibility system contributes still further to the genetical flexibility of the organisms.

Following the fusion of cells two nuclei become associated in a common cytoplasm. However, the fusion of nuclei is delayed until just before meiosis which takes place in basidia. The temporal separation of plasmogamy and karyogamy in a dikaryon is another important factor which contributes to the flexibility of the system. For although the dikaryon is a functional diploid, its two nuclei can be freely exchanged, so that there is a continual introduction of new genetical complements. The newly introduced nuclei can migrate throughout the resident mycelium and successful combinations of nuclei can be selected. Buller (1931) obtained the estimate of 1 mm/h for the rate of migration of the invading nuclei of Coprinus lagopus (=C.radiatus). The rate of migration obtained for P.coprophila in this study was 0.3 mm/h (Watling and Jurand, 1971). In the bifactorial system migration of nuclei can take place as long as the B locus is different, and therefore migration occurs in roughly half of all monokaryotic matings. It was found in this study that migration is sometimes genetically restricted by blocker mutants. 'Blockers' have been found in 5 out of 13 isolates whose incompatibility systems were determined (Table 4.4). It is possible that by preventing the formation of extensive dikaryotic

mycelia, blocker mutants may have some role to play in the initial isolation of the breeding groups.

The initial isolation of breeding groups could also come about by prevention of interbreeding at any stage in the life cycle. It could occur at cell fusion or at heterokaryon formation or at meiosis in the mature fruit body. Hyphal fusion could be prevented by a breakdown in the system of recognition which is known to exist between hyphal cells and between hyphae and oidia. However, hyphal recognition and fusion are not specific to isolates of an interbreeding population, but also occur between isolates of different but related breeding groups. Cell fusion may still take place between these close relatives but the hybrid cells vacuolate and die. Such lethal reactions are characteristic of many crossings in the P.candolleana and P.gracilis complexes. Prevention of heterokaryon formation due to the lethal reaction may have led to the original isolation of the breeding groups. However, the lethal reaction could have developed secondarily, and may now simply maintain the separation of already distinct groups.

Whatever the original act of isolation, the ancestor of a new breeding group must be an isolated mycelium. A monokaryotic mycelium has little chance of survival in competition with the neighbouring dikaryons. It may become dispersed due to the formation of monokaryotic fruit-bodies, but the opportunities for recombination are necessarily limited. Middleton (1964) has shown that meiosis occurs in monokaryotic fruit-bodies of Schizophyllum commune and the same probably applies for Psathyrella. However, though chiasmata must be formed, there can be no effective recombination as long as the diploid cell is the result of fusion of identical nuclei.

An isolated dikaryotic mycelium has more chance of survival and of giving rise to a new population. Even though inbreeding would have to take place at the beginning the evolution of new incompatibility alleles is much more likely in a dikaryotic or heterokaryotic system than in a monokaryotic one. New, non-parental alleles can arise by crossing over between the sub-units of the incompatibility factors (Papazian, 1950). Alternatively, the incompatibility system can be disrupted and self-fertility imposed due to mutants or specific suppressors of the mating type factors (Raper, 1966).

Clearly the evolution of a new breeding group takes place in stages and involves many gene changes. It may never be possible to work out this sequence exactly, but there is little doubt that the evolution of new breeding groups can take place sympatrically due to genetically controlled acts of isolation.

After isolation changes in fruit body morphology may occur due more to chance than selection. The degree of morphological divergence may reflect the time since the breeding group's isolation, though obviously there is no linear relationship. This is the situation now found in Psathyrella. There are many inter-sterile breeding groups in the process of diverging morphologically from one another. Since the morphological discontinuities between breeding groups are not equal, it is not surprising that there is disagreement among the classical taxonomists as to what level of morphological discontinuity should be sufficient for the description of a new morphological species.

The species of the classical taxonomists are described on the basis of morphological evidence only and so can be referred to as

morphospecies (Cain, 1954). Since Psathyrella fruit-bodies are simply constructed and offer few characters for description, even minute differences tend to be given taxonomic importance. Without experimental evidence on the stability of the characters used, some species are bound to be described on the basis of unstable differences. This particularly applies to the narrow species concept favoured by Smith (1968) and Orton (1960). Their policy is to describe a new species whenever a description does not agree convincingly with any species in the available literature. It is because of the narrowness of Smith's species concept that as many as 400 Psathyrella species are described for North America (Smith, 1972). Van Waveren (1971a, 1971b, 1972) in his studies of European isolates uses a wider species concept. It has been one of the aims of this study to compare the species concepts used by these two eminent Psathyrella taxonomists with each other and with the breeding evidence.

Twelve breeding groups were delimited in three sections of the genus using the mycelial crossing test. The presence of clamp connections following the crossing of two monokaryotic mycelia was taken to be evidence that the mycelia belong to the same breeding group. Luckily clamp connections occur in all dikaryotic Psathyrella isolates. In some species of Coprinus clamp connections are never formed and then different tests of crossability have to be used. The units delimited using the clamp formation criterion are here called breeding groups. It is implied that if clamps are formed between two isolates, the isolates are able to interbreed in nature. This assumption is open to two objections. Firstly, clamp formation may not necessarily be followed

by the formation of mature F_1 fruit bodies. Secondly, what happens in the artificial environment of the culture plate does not necessarily hold in the field. The first criticism is difficult to dismiss since most Psathyrella species do not fruit in culture. Only isolates of P.coprophila, Breeding Group I fruited easily in culture and in this breeding group mature F_1 fruit bodies were formed in all inter-isolate crosses. The second criticism becomes less valid when it is considered that geographical isolation is not very effective in this group of fungi and, in many isolates, the basidiospores are capable of germinating even in distilled water.

The breeding groups delimited in this study are listed in Table 11.1 together with the number of isolates and their country of origin. A few of the breeding groups include isolates from several different countries; some of the P.candolleana breeding groups include both European and American material. A similar case of interfertility between geographically distant isolates was found by Lange (1952) in Coprinus C.subimpatiens but the results of Vandendries (1927) on the geographical distribution of interfertile isolates of C.micaceus are different. He found intersterility to be the rule when confronting strains from localities several hundred kilometers apart.

Table 11.1 also shows that breeding groups belonging to the morphological complex can exist side by side in the same country. In P.candolleana two different breeding groups were found growing in the same habitat. This means that the hyphae of related breeding groups must be able to recognise self from non-self if the breeding groups are to remain isolated. The homing and fusion of hyphae with oidia followed by the vacuolation and death of the hybrid cells shows how this may take place.

Table 11.1 Number of isolates and their country of origin in each of the breeding groups delimited in the study.

Breeding group		Country						
		En	Sc	Fi	Ho	Sw	U.S.A.	Total
P.candolleana	I	1		1			15	17
	II	3		1			1	5
	III						1	1
	IV							1
P.gracilis	I		6					6
	II	3	8		2			13
	III	1	6					7
P.coprobia	I		2					2
	II	2	3		1			6
	III		1					1
P.coprophila	I		4			2		6
	II		1					1

Countries: En - England, Sc - Scotland, Fi - Finland, Ho - Holland
Sw - Sweden.

The existence of groups such as these, "adjacently or biotically sympatric, without crossbreeding or forming hybrids is considered to be a definitive qualification for the naming of different species" (Mettler & Gregg, 1969). By this definition a breeding group is synonymous with a species. This is the view accepted by Kemp (personal communication) in Coprinus, and by Nelson (1963) for the fungi as a whole. The question of whether a breeding group is really a species or not can be reduced to one of semantics but it is more important than that. To the taxonomist, the species must be morphologically distinguishable, and have a unique name and type. At the moment, the biological species definition cannot be applied in Psathyrella, without adopting a radically different approach.

In Psathyrella there is often little morphological differentiation between related breeding groups, for example between the four P.candolleana breeding groups, and two of the P.gracilis breeding groups. Similar results for the P.candolleana complex were reported by Galland (1973) although the identity of her breeding groups with those delimited in this study was not tested. Romagnesi suggested that the four P.candolleana breeding groups delimited by Galland be given four names: P.candolleana, P.elegans, P.proxima and P.scotospora. The use of such different and unrelated names which give no indication of morphological similarity, is not helpful.

Secondly each species must have a type. The type is commonly placed in a recognized herbarium and its description must be published in Latin in a form available to the public. If each of the breeding groups here described were to be defined as a species, the type would have

to be not a desiccated fruit-body but a living culture in a culture collection, preferably for a tetrapolar species, four living cultures to represent each of the four mating types. Unfortunately cultures in collections often die, and those that survive may mutate with time. Should anybody want to identify an isolate to the species level, they would have to order subcultures of the original type culture to be made, and then check the identity by experiment. The difficulty of such a procedure would probably discourage identification altogether.

There is yet a third disadvantage in accepting the biological species definition for the genus as a whole. Only about half of all Psathyrella isolates can be grown in culture and for the rest one would have to content oneself with the old morphological species definition. The result is likely to be a genus of "unequal species" unless some indication is given in the name, of the concept used.

As it seemed that neither the biological nor the morphological species concept by itself could give a complete or universal answer in Psathyrella an attempt was made fo find a compromise solution. As a first step the extent to which the breeding groups could be separated on morphological grounds was investigated.

Several of the morphological characters varied as much within as between the breeding groups, for example fruit-body colour, gill colour, cystidium size, suggesting against the use of these characters as a basis for the description of new taxa. The characters of basidiospore size and cystidium shape were less variable within the breeding groups and therefore make more reliable diagnostic characters.

Between the four P.candolleana breeding groups and between two of the three P.gracilis breeding groups little morphological differentia-

tion was found. Similar examples of intersterility without correlated morphological differences have also been recorded by other authors in other genera: Boidin (1951) in Gloeocystidium tenue, Lange (1952) in Coprinus subimpatiens, Burnett and Boulter (1963) in Mycocalia denudata. In the fruit-fly Drosophila similar inter-sterile units have been referred to by Dobzhansky (1937) as "sibling species".

Some morphological differences were recorded between the coprophilous breeding groups but too few isolates were examined to allow definite taxonomic conclusions to be made. P.gracilis Breeding Group II differed from the other two breeding groups only slightly, but after some statistical tests it was decided that it represents a different morphospecies, P.microrrhiza.

To compare the different morphospecies concepts adopted by Smith and van Waveren, herbarium material of the same six P.gracilis complex fruit-bodies were sent for identification. The results of the correspondence showed that two entirely different species concepts were chosen by these two taxonomists (Table 8.6). The narrow morphospecies of Smith was even narrower in its limits than the breeding group and there was no correlation between his interpretation of the morphological differences and the breeding evidence. The inevitable practical difficulties that arise from the use of a narrow concept cannot therefore be justified, at least in this group of isolates, and the same may apply to the rest of the 400 species which Smith recognizes in the U.S.A. The morphospecies concept adopted by van Waveren resulted in species wider than the breeding group and on the whole there was good correlation between their limits and sets of closely related breeding groups.

The same morphological evidence has obviously been used by these two taxonomists to produce radically different classifications, and it has been possible to test the merits of the two classifications using the breeding evidence. The evidence shows that the wide-morpho-species reflect better the fundamental discontinuities between taxa. It is therefore suggested that in general when no breeding information is available the morphospecies should be wide so as to make possible easy identification using morphological criteria. Where breeding information is available the morphospecies can be split up into breeding units or biological species.

Thus a taxon can be defined as a morphologically discrete group of individuals, a genetically discrete group of individuals, or a morphologically and genetically discrete group of individuals. The difficulty arises in assigning names because the present code of nomenclature requires a decision about which is the fundamental unit or species. Gilmour and Gregor (1939) suggested the deme terminology in an attempt to resolve situations such as these. Gilmour and Heslop-Harrison (1954) later developed it further. In this system the morphospecies fills the needs of a broad-purpose classification whereas the 'demes' are units of micro-evolutionary change. The suffix 'deme' is neutral and can only be used with a more or less self-explanatory prefix, e.g. pheno-, topo-, eco-, gamo- etc. used singly or in combination, to indicate the particular process of micro-evolution involved. For example, P.gracilis (Breeding Groups I and III) could be referred to as gamodemes, whereas Breeding Group II = P.microrrhiza could be referred to as a phenogamodeme. But the deme terminology does not, and was never intended to, offer any

definite or uniform method of naming individual examples. This may be the main reason why it has only rarely been used.

The deme terminology was adopted by Duncan and Macdonald (1967) in Auricularia auricula but in that case it was the ecodemes that were the units of micro-evolutionary change. Ecological units are more difficult to typify and the deme terminology is therefore more useful. In the case of Psathyrella a definite system of nomenclature is needed which makes easy reference possible. It is therefore suggested that the broad morphospecies be given the simple binomial e.g. P.candolleana and the breeding groups or biological species be denoted by a Greek letter prefix, e.g. P.α candolleana, P.β candolleana, P.γ candolleana etc. The breeding groups delimited in this study can therefore be renamed as follows:

P.candolleana	I	=	P.α candolleana
"	II	=	P.β candolleana
"	III	=	P.γ candolleana
"	IV	=	P.δ candolleana
P.gracilis	I	=	P.α gracilis
"	II	=	P. microrrhiza
"	III	=	P.β gracilis
P.coprobia	I	=	P.α coprobia
"	II	=	P.β coprobia
"	III	=	P.γ coprobia
P.coprophila	I	=	P.α coprophila
"	II	=	P.β coprophila (?)

One advantage of using the Greek letter system instead of the breeding group number is that the biological species name is in the form

of the binomial. Thus both the biosystematic and the conventional taxonomists should be satisfied. Secondly, an indication of morphological similarity is given. Thirdly the system is expandible; as more breeding information becomes available more 'bio'-species can be added.

In some cases it may be possible to work out a hierarchy above the 'bio'-species level using information on the reaction between hyphae and oidia. Three different responses have been found and more may be revealed in further studies. Fusion between hyphae and oidia is easier to detect than is the fusion of two hyphal cells, since the directed growth of hyphae towards oidia is clearly visible microscopically. Obviously, the ability of cells to fuse is an indication of closer affinity than if no fusion takes place at all. The events which follow fusion suggest further levels of relationship. For example, in the P.gracilis complex homing followed by the lethal reaction took place between Breeding Groups I and III but not between Breeding Group II and the other two breeding groups. The interpretation that Breeding Groups I and III are more closely related than either is to Breeding Group II is corroborated by the morphological evidence.

In this study breeding groups were delimited in only about one tenth of the genus. However the breeding evidence made possible not only the definition of twelve biological species, it also showed a way of judging the soundness of morphological classifications and the reliability of several characters on which taxa are often based. In this way a few conclusions were made which apply to the genus as a whole and perhaps to other agaric genera as well.

Romagnesi is supposed to have written in a letter to van Waveren

(unpublished): "Je me demande si une monographie des Drosophila (he uses the old generic name) est humainement possible à réaliser dans l'état de la science d'autant que toutes les espèces n'ont pas des spores germant en culture pure". This is perhaps an overstatement. This study has demonstrated the value of experimental evidence in the understanding of evolutionary processes and in producing a more objective classification in this morphologically difficult genus of the higher fungi.

APPENDIX I

List of all isolates obtained in culture together with those in which attempts at culturing failed.

Culture code numbers are only given to isolates successfully cultured. Isolates belonging to the same breeding group are indicated by the ditto sign ("). The exact place of collection and habitat are not given, but can be found on the herbarium packet labels. All the isolates collected by myself will be placed in the herbarium of the Royal Botanic Garden, Edinburgh. All the European isolates listed will then be available for reference in the above herbarium. The American isolates, from which spore prints were received, are being kept in the Michigan Herbarium.

The age of the fruit-body at the time of spore plating is given since it could, in some cases, be responsible for the failure of spore germination. The isolates which produced fruit bodies in culture are marked * by the culture code number. The stocks currently held in culture (October, 1974) are indicated in the column of "stocks held". Unless otherwise stated the stock cultures are monokaryotic mycelia.

Collectors' Initials:

A.B.- Angela Ballantyne, Edinburgh; J.C.- Jeffrey Collman, Devon; J.D.- J. Daams, Leiden, Holland; F.H. - Florence Hoseney, Michigan,U.S.A.; M.J. - myself; R.N. - Rosemary Nimke, Michigan, U.S.A.; P.O. - Peter Orton, Perthshire; S.P. - Stanley Porter, Birmingham; M.Ri. - Michael Richardson, M.Ro.- Maurice Rothero, Birmingham; R.W. - Roy Watling, Edinburgh; K.vW. - Kits van Waveren, Amsterdam, Holland.

APPENDIX I

Approximate Identification By Morphology	Place and Year of Collection	Collector and No.	Age in months of spores plated	Culture Code No.	No. of stocks held
P.ammophila	Inverness-shire, 1970	R.W.	0	44	1
"	East Lothian, 1967	R.W.	26	-	-
P.atomata	Perthshire, 1967	R.W.	26	-	-
P.atomata	Peebles-shire, 1971	M.J. 82	2	82	1
"	Yorkshire, 1971	M.J. 98	2	98	1
"	" , 1971	M.J.102	2	102	1
P.candolleana I	Michigan, U.S.A.,1971	R.W.6609	3	24	-
"	Herefordshire,U.K.,1971	M.Ro.(M.J.141)	2	141	4
"	Turku, Finland, 1971	R.W.8412	2	145	1
"	Michigan, U.S.A., 1971	A.S.81096	1	160	4
"	"	A.S.81097	1	161	-
"	"	A.S.81098	1	162	-
"	"	A.S.81099	1	163	3
"	"	A.S.81103	1	165	1
"	"	A.S.81112	1	166	-
"	"	F.H. 1974	1	167	2
"	"	A.S.81113	1	168	-
"	"	R.N. 204	1	169	3
"	"	R.N. 208	1	170	4
"	"	A.S. -	1	173	-
"	"	A.S.81153	1	175	-
"	"	A.S.81156	1	176	1
"	"	F.H. 2194	1	179	2
P.candolleana II	Yorkshire, 1970	R.W. 7560	4	42	-
"	Surrey, 1969	P.O. 3399	18	43	-
"	Warwickshire, 1971	S.P. 575	3	136	2
"	Turku, Finland, 1971	R.W. 8375	2	142	2
"	Helsinki, Finland,1971	R.W. 8561	2	146	-
"	Ohio, U.S.A., 1971	F.H. 2096	1	171*	1

APPENDIX I - continued

Approximate Identification By Morphology	Place and Year of Collection	Collector and No.	Age in months of spores plated	Culture Code No.	No. of stocks held
P.candolleana	Michigan, U.S.A. 1972	F.H. 2194	1	178	1 dik
P.caput-medusae	Perthshire, U.K. 1970	M.J. 66	1	-	-
"	Stirlingshire, 1970	R.W.	1	-	-
"	Perthshire, 1970	R.W. 7518	2	41	1
P.cernua	Perthshire, 1969	M.J. 29	1	-	-
P.conopilea	Yorkshire, 1971	M.J. 96	3	96	1
"	Midlothian, 1971	M.J. 107	2	107	-
P.coprobia I	Mull, 1968	R.W. 7814	27	30	2
"	Mull, 1968	R.W. 7843	26	31	3
P.coprobia II	Hilversum, Holland, 1970	J.D.(M.J.32)	9	32	2
"	Perthshire, 1970	M.J. 33	3	33*	2,1 dik
"	Mull, 1968	R.W. 7756	16	40*	-
"	Devon, 1971	M.J. 87	2	87	3,1 dik
"	Devon, 1971	M.J. 88	2	88	1
"	Perthshire, 1971	A.B.(M.J.133)	2	133	-
P.coprobia III	Midlothian, 1966	R.W. 5243	39	14*	8
P.coprophila I	Midlothian, 1966	R.W. 3947	40	1*	2
"	Perthshire, 1967	R.W. 7440	27	51*	1
"	Midlothian, 1971	M.J. 84	2	84*	5
"	Uppsala, Sweden, 1971	R.W.(M.J.128)	2	128*	-
"	Uppsala, Sweden, 1971	R.W.(M.J.129)	2	129*	-
"	Perthshire, 1967	R.W. 5069	47	144*	-
P."fimetaria" = P.coprophila II	Perthshire, 1970	M.J.(R.W.7470)	2	59	4
P.flexispora	East Lothian, 1967	R.W. 5244	28	-	-
P.gossypina	St.Kilda, 1967	R.W. 6227	28	-	-
"	Midlothian, 1971	M.J. 106	0	106	1

APPENDIX I - continued

Approximate Identification By Morphology	Place and Year of Collection	Collector and No.	Age in months of spores plated	Culture Code No.	No. of stocks held
P.gracilis I	Edinburgh, 1969	M.J. 3	2	3	2
"	Perthshire, 1970	M.J. 55	2	55	1
"	Perthshire, 1970	M.J. 58	2	58	1
"	Perthshire, 1970	M.J. 60	2	60	1
"	Midlothian, 1971	M.J. 110	1	110	2
"	East Lothian, 1971	M.J. 111	1	111	1
P.gracilis II = P.micorrhiza	Midlothian, 1969	M.J. 15	3	15	2
"	Edinburgh, 1969	M.J. 17	3	17	-
"	Edinburgh, 1970	M.J. 67	1	67	1
"	Edinburgh, 1970	M.J. 68	1	68	-
"	Edinburgh, 1970	M.J. 70	2	70	-
"	Edinburgh, 1970	M.J. 71	2	71	-
"	Edinburgh, 1970	M.J. 73	2	73	-
"	Inverness-shire, 1971	M.J. 117	1	117	1
"	Devon, 1971	J.C.(M.J.121)	1	121	4
"	Warwickshire, 1971	S.P. 620	1	151	1
"	Delden, Holland, 1971	K.vW. 30	1	152	-
"	Delden, Holland, 1971	K.vW. 23	1	153	-
"	Warwickshire, 1971	S.P. 658	1	156	1
"	Warwickshire, 1971	S.P. 649	1	157	1
"	Warwickshire, 1971	S.P. 664	1	158	1
P.gracilis III	Edinburgh, 1969	M.Ri.(M.J.16)	3	16	1
"	Edinburgh, 1970	M.J. 72	1	72	1
"	Edinburgh, 1970	M.J. 74	1	74	1
"	Yorkshire, 1971	M.J. 101	2	101	4
"	Falkirk, 1971	M.J. 104	2	104	1
"	Falkirk, 1971	M.J. 105	2	105	1
"	East Lothian, 1971	M.J. 112	2	112	-

APPENDIX I - continued

Approximate Identification By Morphology	Place and Year of Collection	Collector and No.	Age in months of spores plated	Culture Code No.	No. of stocks held
P.hirto-squamosa	U.S.A., 1969	R.W.	1	-	-
P.hydrophila	Midlothian, 1969	M.J. 43	1	-	-
P.hydrophila	Huntingdonshire, 1969	P.O.	9	-	-
P.hydrophila	Michigan, U.S.A. 1969	B.W. 6965	1	-	-
P.hydrophila	Perthshire, 1969	M.J. 30	1	-	-
P.hydrophila	Norfolk, 1968	P.O.	15	26	-
P.hydrophila	Midlothian, 1971	M.J. 109	tissue	109*	1 dik
P.leucotephra	Perthshire, 1969	R.W. 7665	6	-	-
P.leucotephra	Warwickshire, 1971	S.P. 135	1	-	-
P.marescibilis	Perthshire, 1970	P.O.	2	46	1
P.microlepidota	Norfolk, 1967	P.O.	30	-	-
P.multipedata	Warwickshire, 1971	S.P.(M.J.140)	2	-	-
P.nolitangere	St.Kilda, 1967	R.W. 6228	26	-	-
P.obtusata	Yorkshire, 1968	R.W. 5974	14	5*	1 dik
P.obtusata	Nairnshire, 1967	R.W.	30	-	-
P.obtusata	Perthshire, 1970	M.J. 62	2	62	-
P.orbitarum	Inverness-shire, 1969	P.O. 3752	9	25	1
P.pillulaeformis	Mull, 1968	R.W. 6046	14	-	-
P.pseudogracilis	Yorkshire, 1970	R.W. 7572	-	38	-
P.pygmaea	Yorkshire, 1971	M.J. 94	2	94	1
P.rigidipes	Michigan, U.S.A.1969	R.W.	1	-	-
P.sarcocephala	Michigan, U.S.A.1969	R.W.	1	-	-
P.sarcocephala	Michigan, U.S.A.1969	R.W.	1	11	-
P.sarcocephala	Somerset, 1969	P.O. 3760	6	-	-
P.semivestita	Surrey, 1969	P.O. 3761	6	53	-
P.spadiceo-grisea	Yorkshire, 1967	R.W. 5018	14	4	1
P.spadiceo-grisea	Perthshire, 1969	M.J. 8	1	8	2
P.sphagnicola	Perthshire, 1968	R.W. 5155	15	-	-
P.squamosa	Perthshire, 1969	M.J. 28	1	-	-
P.squamosa	Perthshire, 1969	M.J. 29	1	-	-
P.squamosa	Edinburgh, 1969	M.J. 22	1	-	-

APPENDIX I - continued

Approximate Identification By Morphology	Place and Year of Collection	Collector and No.	Age in months of spores plated	Culture Code No.	No. of stocks held
P.stellata	Devon, 1969	P.O. 3763	11	-	-
P.stipitissima	Yorkshire, 1971	M.J. 93	1	-	-
P.subnuda	Mull, 1968	P.O.	18	-	-
P.tephrophylla	Surrey, 1968	P.O. 3407	18	-	-
P.typhae	Yorkshire, 1967	R.W. 5001	27	-	-
P.vernalis	Yorkshire, 1967	R.W. 5004	27	-	-
P.vernalis	Midlothian, 1970	M.Ri.(M.J.18)	0	18	-
P.xanthocystis	Mull, 1969	P.O. 3764	11	49	-
Unidentified sp.	Midlothian, 1970	M.J. 49	1	49	-
"	Midlothian, 1970	M.J. 50	1	50	-
"	Perthshire, 1970	M.J. 57	1	57	-
"	Midlothian, 1971	M.J. 80	2	80	-
"	Midlothian, 1971	M.J. 81	2	81	-
"	East Lothian, 1971	M.J. 115	2	115	-
Lacrymaria velutina	Edinburgh, 1969	M.J. 27	tissue	27	1 dik
	Midlothian, 1970	M.J. 52	tissue	52	-
"	Edinburgh, 1970	M.J. 75	tissue	75	-
"	Midlothian, 1970	M.J. 77	tissue	77	-

APPENDIX II

Listo of cultures sent to recognised culture collections

Centraalbureau voor Schimmelcultures, Baarn, Holland.

American Type Culture Collection (A.T.C.C.), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.

Identification	Culture No.	Centraalbureau	A.T.C.C.
P.ammophila	44	1 dik.	-
P.conopilea	96	1 mono., 1 dik.	-
P.coprobia	33	mating types	-
P.coprobia	14	1 mono., 1 dik.	-
P.coprophila	1	mating types, 1 dik.	mating types, 1 dik.
P."fimetaria" = P.coprophila II	89	mating types, 1 dik.	mating types, 1 dik.
P.obtusata	5	3 monos., 1 dik.	-
P.pseudo-gracilis	38	1 dik.	-

APPENDIX III

Spore length measurements (in μm) in the P.gracilis group of isolates. The Isolate Numbers are indicated at the head of each column.

Breeding Groups I and III

3	58	60	16	72	74
12.4	12.8	12.6	13.2	13.0	13.8
13.0	13.0	12.4	12.0	12.0	12.6
13.2	12.4	12.6	12.4	13.4	13.0
12.2	12.8	12.2	12.8	13.2	12.8
12.6	12.4	12.4	12.8	14.4	13.0
12.4	13.0	12.6	12.6	13.4	12.2
11.5	13.0	12.4	13.2	12.8	13.0
13.2	13.0	12.8	12.6	12.6	14.2
11.5	13.0	12.8	12.2	12.0	13.2
13.6	13.6	12.2	12.6	13.0	13.2
13.0	13.0	12.0	12.2	13.8	12.8
12.8	13.2	13.2	12.8	12.6	12.6
12.4	12.2	12.6	13.2	14.6	12.2
12.0	13.2	12.6	13.4	13.2	12.6
12.4	14.0	12.4	14.2	12.2	13.4
12.4	14.9	13.4	15.0	13.6	12.6
12.4	15.1	11.8	12.6	13.2	12.6
11.5	12.8	12.2	13.4	12.8	13.2
13.2	12.0	13.0	14.4	13.8	12.0
12.8	12.0	12.8	14.4	13.0	12.8

Breeding Group II

67	68	70	71	73	121	151
12.0	11.0	11.4	13.4	13.0	12.6	11.0
11.5	11.2	11.0	11.8	11.0	11.4	11.8
11.5	11.4	11.6	12.4	12.2	12.6	11.4
11.0	11.0	11.4	12.2	12.2	11.0	11.8
11.4	11.2	12.8	11.8	12.2	11.8	11.4
12.0	12.2	11.4	12.8	12.0	10.4	11.4
11.8	11.0	11.0	12.2	10.4	12.2	11.8
10.8	10.8	10.6	12.2	12.6	12.6	12.2
10.8	11.6	11.4	11.2	10.6	13.8	11.1
11.4	11.4	11.8	11.6	12.0	11.8	11.0
11.8	11.2	10.6	12.4	12.6	12.6	11.6
11.2	11.4	10.8	11.6	11.4	12.4	11.0
11.5	11.2	12.2	11.2	11.8	11.4	11.4
11.4	11.2	11.0	13.2	12.4	12.2	11.0
11.4	10.6	12.2	10.4	12.4	11.8	11.4
10.8	10.6	10.8	11.8	11.0	11.8	11.4
11.5	11.0	11.0	11.8	10.8	11.8	11.4
12.8	11.4	11.0	11.8	10.6	11.6	11.4
11.5	11.8	10.2	12.2	12.4	11.8	11.0
12.0	11.0	11.4	11.6	11.4	11.4	11.0

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SHORT COMMUNICATIONS

Surface Ultrastructure of Oidia in the Basidiomycete *Psathyrella coprophila*

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The existence of oidia has long been known and their structure and development have been described using the light microscope (Brodie, 1936). They are formed asexually, are uninucleate and usually borne in clusters on the monokaryotic mycelia of many basidiomycetes. Oidia of different species may have different functions. In some species they readily germinate to form monokaryotic mycelia. In many coprophilous species they do not germinate (Falck, 1902), and they probably act as fertilizing agents or spermatia. The oidia of *Psathyrella coprophila* Watling (Watling & Jurand, 1971), which were used in this investigation, do not germinate. They are borne on oidiophores and cohere in a droplet of mucilage.

The surface structure of oidia is of interest in studies of the relationship between the hyphae and the oidia. Hyphae of many species are known to react to an oidial stimulus by growing towards and fusing with oidia (Bistis, 1970; Kemp, 1970). The directed growth and fusion between hyphae and oidia is either followed by dikaryotization or by vacuolation and death. Dikaryotization indicates that two isolates belong to the same species. Homing followed by a lethal reaction indicates that two isolates are closely related. The physiological basis for the recognition of oidia by hyphae has not yet been resolved and a study of the oidial surface might be relevant to this problem.

From this preliminary study it is suggested that the surface of the oidia of *Psathyrella coprophila* has numerous filamentous appendages which may be embedded in a layer of mucilage. A similar surface structure has been described in certain bacteria. In some respects the filamentous appendages appear to resemble bacterial fimbriae (pili) (Duguid, Smith, Dempster & Edmunds, 1955; Brinton, 1965), and the mucilaginous coat in which they are embedded may be similar to bacterial capsules (Duguid, 1951).

METHODS

Psathyrella coprophila was chosen because it produces abundant oidia in culture. Monokaryotic mycelia, from basidiospores of the type specimen which is deposited in the herbarium of the Royal Botanic Garden, Edinburgh, were grown on agar plates at 20 °C on horse-dung extract agar (Lange, 1952). The oidia were harvested when the mycelium almost covered the agar surface.

For electron-microscope preparations the oidia were harvested with 0·81 % (w/v) NaCl. About 4 ml of the saline solution was poured into each plate, the oidia suspended by gentle rocking and the suspension centrifuged at 3000 rev./min for 5 min to concentrate the oidia into a pellet. Three fixation methods were used:

(i) *Fixation with* OsO_4 (1 %, *w/v*) according to Palade (1952), but with addition of 0·045 mg sucrose/ml of fixative, for 1 h at room temperature;

(ii) *Double fixation with glutaraldehyde and OsO_4.* The oidia were fixed for 1 h at 0 to 2 °C with glutaraldehyde (2·5%, v/v) in 0·1 M-phosphate buffer at pH 7·2, then washed with 0·1 M-phosphate buffer and fixed with OsO_4 (1%, w/v) dissolved in the buffer;

(iii) *Double fixation in the presence of ruthenium red.* The fixation procedure used was that of Luft (1965). It has been described in detail by Pate & Ordal (1967).

After fixation by methods (ii) and (iii) the pellets were rinsed with buffer, dehydrated with a graded series of alcohol solutions, passed through 1,2-epoxy propane and embedded in Araldite. Fixation method (i) was followed, without rinsing, by dehydration and embedding. Sections were prepared with glass knives on an LKB Ultratome. Sections of the osmium-fixed material were stained with 2·5% (w/v) uranyl acetate and 1% (w/v) potassium permanganate for 20 min (Lawn, 1960). The sections of the double-fixed materials, (ii) and (iii), were stained with 1% (w/v) uranyl acetate solution for 30 min and with lead citrate solution (Reynolds, 1963) for 15 min. The stained sections were examined with an AEI EM6B electron microscope.

For light-microscope observations the oidia were suspended in distilled water, concentrated by centrifugation and relief stained with indian ink (Duguid, 1951).

RESULTS

Sections of oidia prepared after fixation with OsO_4 and those prepared after double fixation showed filamentous appendages on the surface of the oidia (Fig. 1 *a*, *b*). The filaments were similar in size and shape after both (i) and (ii) methods of fixation. Their diameter and length were approximately 10 nm and 0·5 μm respectively and they appeared to taper slightly. The structure of the appendages differed after the two fixation methods. After osmic fixation the filaments seemed to be hollow while those after double fixation appeared solid and more heavily stained. Also in the osmic-fixed material some of the filamentous appendages seemed to pass through the oidial wall and the plasmalemma but this was never seen in the double-fixed material.

Fig. 1 *a*, *b* clearly indicate the presence of the filamentous appendages but give no indication of an extracellular capsule. In the indian ink preparations examined by the light microscope the capsule was seen as a light band surrounding the oidia (Fig. 1 *c*). The capsule was similar to those found in many bacteria (Duguid, 1951). The ruthenium-red preparations also indicated the presence of a capsule (Fig. 1 *d*). The region positive for ruthenium red that surrounded each oidium was similar in width to the light band which was seen in the light microscope preparations and to the length of the filaments seen by the fixation techniques (i) and (ii). A fibrillar component was also present in the ruthenium-red positive region but there were no distinct appendages. The capsule probably contained some highly polymerized acid mucopolysaccharides because the affinity of ruthenium red to these surface carbohydrates is well documented (Luft, 1964).

Of the internal structures of the oidia only the major organelles were clearly distinguishable. A single, large nucleus occupied almost half of the cell section. All the cell membranes and especially the nuclear envelope were most clearly shown after osmic fixation. The mitochondria were large in proportion to the size of the oidium, and were elongated and few in number. All the oidia contained at least one prominent inclusion which was also visible in the light microscope. These inclusions were probably lipid structures of the type referred to by Hawker (1965).

The final shape of the oidia indicates the method of their formation. Some of them had one flat and one rounded end (Fig. 1 *a*), and others had two flat ends (Fig. 1 *b*). The flat ends

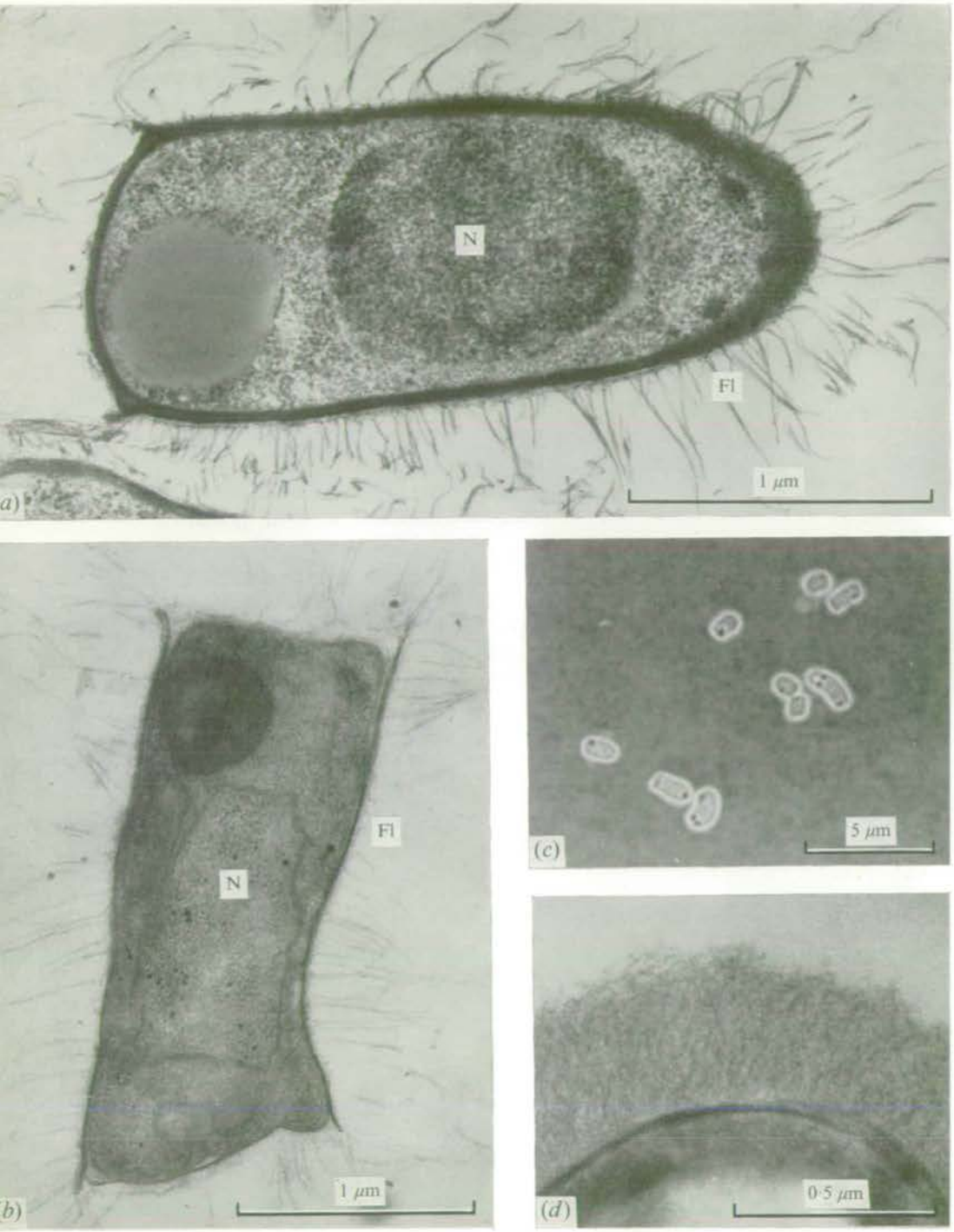

Fig. 1 (*a*) Electron micrograph of a longitudinal section of an oidium double-fixed in glutaraldehyde osmic acid, showing filaments (Fl), and nucleus (N).

(*b*) Electron micrograph of a longitudinal section of an oidium fixed in osmic acid only, showing filaments (Fl) and nucleus (N).

(*c*) Photomicrograph of oidia relief stained in indian ink, showing capsule.

(*d*) Electron micrograph of a section of the capsule region of oidium double fixed with glutaraldehyde and osmic acid in the presence of ruthenium red.

had terminal flanges. Oidia of the first type were produced as the terminal sections of the finger-like outgrowth of the oidiophore, while the others were intercalary.

DISCUSSION

Several different techniques were used in order to reveal the surface structure of oidia but the relationship of the filamentous appendages to the capsule is still not clear. No single preparation has yet shown the filamentous appendages together with the capsule.

There are two possible explanations of the results presented in this communication. Either the filaments were true filamentous organelles present in the living organism, or they were artifacts produced during the fixation or dehydration of the capsule. There is some evidence which argues against the possibility of artifacts. Two different fixation techniques give comparable results (Fig. 1 *a*, *b*), and although dehydration of the capsule could lead to the formation of filaments, it is doubtful that such regularity in form and thickness could be achieved by simple drying. There was some indication that in a few places filaments passed through the oidial wall and plasmalemma into the oidium, but the relevant micrographs lacked contrast and would not reproduce satisfactorily. Perhaps the failure to show a clear continuation of the filaments through the wall is due to their small diameter in relation to the thickness of the sections.

The observations of the surface structure presented here are by no means unique. Similar observations and difficulties in interpretation have been described in many bacteria. The term fimbriae which was originally used to describe rigid, hairlike appendages on the surface of many capsulate and non-capsulate bacteria (Duguid *et al.* 1955) has now been broadened to include branches and flexuous filaments (Yanagava & Otsuki, 1970; Wistreich & Baker, 1971). Some of these strongly resemble the filamentous appendages described in this report.

Bacterial fimbriae are usually made of single proteins and often possess antigenic properties. The chemical nature of the oidial filamentous appendages is not yet known and further work is necessary before more certain interpretations of this work can be put forward.

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An incompatibility system determined by three factors in a species of *Psathyrella* (Basidiomycetes)

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SUMMARY

Evidence is given for a trifactorial system of incompatibility in an isolate identified as *Psathyrella coprobia*. The three factors are designated A, B and C. They are thought to be inherited independently. The function of factor A is most probably the same as that of factor A of bifactorial species. Factor B is concerned with the initiation of fruit body primordia, while all three factors must be heterozygous for the occurrence of nuclear migration and the formation of mature fruit bodies.

1. INTRODUCTION

Incompatibility in fungi was first described by Blakeslee (1904) in *Rhizopus nigricans*. He used the term 'heterothallism' to describe the situation where sexual reproduction can only take place between hyphae of genetically different mycelia. In this species as in many other phycomycetes the incompatibility system is determined at a single incompatibility locus with two alleles. The opposite of 'heterothallism' is 'homothallism' and this indicates that the sexual cycle can be completed by a mycelium derived from a single spore.

Heterothallism in the basidiomycetes was first described by Bensaude (1918). She found that the incompatibility system in the basidiomycete *Coprinus cinereus* (= *C. lagopus sensu* Buller) was determined at two incompatibility loci both with multiple alleles. Incompatibility of this type is generally referred to as 'tetrapolar' following the initial use of the term 'tetrapolar sexuality' by Bauch (1930) in *Ustilago longissima*. Some basidiomycetes such as *Ustilago violacea* (Burgeff, 1920) were found to have a single factor with multiple alleles. This system is known as 'bipolar incompatibility', though Burgeff had initially used the term 'bipolar sexuality'.

The terms 'incompatibility factor' and 'incompatibility locus' are used synonymously in most of the literature. The term 'factor' is now preferred as it is known that the mating-type determinants consist of at least two subunits which may not always be closely linked (Papazian, 1950; Takemaru, 1957). Since Quintanilha (1939) specifically referred to the incompatibility factors of *Coprinus radiatus* (= *C. fimetarius*) the use of the terms 'unifactorial' and 'bifactorial' have been

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replacing the terms 'bipolar' and 'tetrapolar'. In an incompatibility system determined by three factors the term 'trifactorial' is probably preferable to 'octopolar'.

In a unifactorial system the single factor is designated A, and in a bifactorial system the two factors are designated A and B. In no species are the A and B factors linked. In a bifactorial system heterozygosity for the A factor is responsible for most stages of clamp formation. Heterozygosity for the B factor alone is sufficient to permit nuclear migration (Fulton, 1950). As heterozygosity for both factors is necessary for the formation of complete clamp connexions and for the development of mature fruit bodies the incompatibility mechanism involves some form of complementation.

Basidiospores obtained from the same fruit body are of two mating types in a unifactorial species and of four mating types in a bifactorial species. Each monokaryon can mate with half of the sister monokaryons in a unifactorial species but with only a quarter of the sister monokaryons in a bifactorial species. This restriction in the level of inbreeding is presumably of evolutionary advantage to the species. The presence of a third factor would reduce sister mating even further to one in eight. In theory there is no reason why a trifactorial system should not exist. The results described below show that such a system of incompatibility does exist in an isolate of the genus *Psathyrella*.

2. THE METHOD OF DETERMINING MATING TYPES

The number of mating types present among the basidiospores obtained from a single fruit body is relatively easy to determine by mating a randomly selected sample of monokaryotic mycelia in all combinations, and scoring the matings for the extent of dikaryotization. Reciprocal dikaryotization of the mated mycelia is detected by the presence of clamp connexions throughout both mycelia. It indicates that the two mycelia are fully compatible. Absence of clamp connexions throughout indicates that the two mycelia have incompatibility alleles in common. In bifactorial isolates there is a third class of mating in which clamps can be isolated from the junction line only. Careful examination of the clamps may reveal that they are incomplete or false clamps, but in determining mating types it is necessary to test for nuclear migration as well as for clamps (Aschan, 1954). This can be done by placing the initial inocula some distance apart and by taking sample plugs from the edges of the mycelia as well as from the junction line. If sample plugs are taken from the junction line only it is possible to score a heterokaryotic mycelium with false clamps as a compatible dikaryotic mating because no information on nuclear migration is available. Examples from the literature where incompatibility systems have been wrongly determined because false clamps have been scored as normal clamps are mentioned in the discussion.

3. MATERIAL AND METHODS

All cultures were grown on horse-dung extract medium (LH) following the recipe of Lange (1952) and incubated at 25 °C. In the mating-type determination experiment matings were made on LH agar, four per plate, placing the inocula about 1 cm apart. The inocula were incubated for 5 days before the matings were tested for clamps and nuclear migration. Inocula were initially isolated from the junction line and at 1 cm intervals on both sides, making a total of five samples. In later tests it was found adequate to sample from the junction line and at a distance of 1 cm on each side.

In the growth-rate experiment small plugs of mycelia were taken from the junction line of each mating, were inoculated four per plate on LH, and were incubated for 4 days. The diameter of each colony was measured in millimetres.

In the experiment involving fruit-body formation, plates were inoculated with nine monokaryons in the form of a 3×3 square. The four corner inocula and the central one were of one mating type and the middle inoculum on each of the four sides was of the other type. The plates were incubated in the dark until the mycelia had met and were then transferred into the light at a room temperature of 20 °C. They were examined daily for the formation of primordia and mature fruit bodies over a period of 4 weeks.

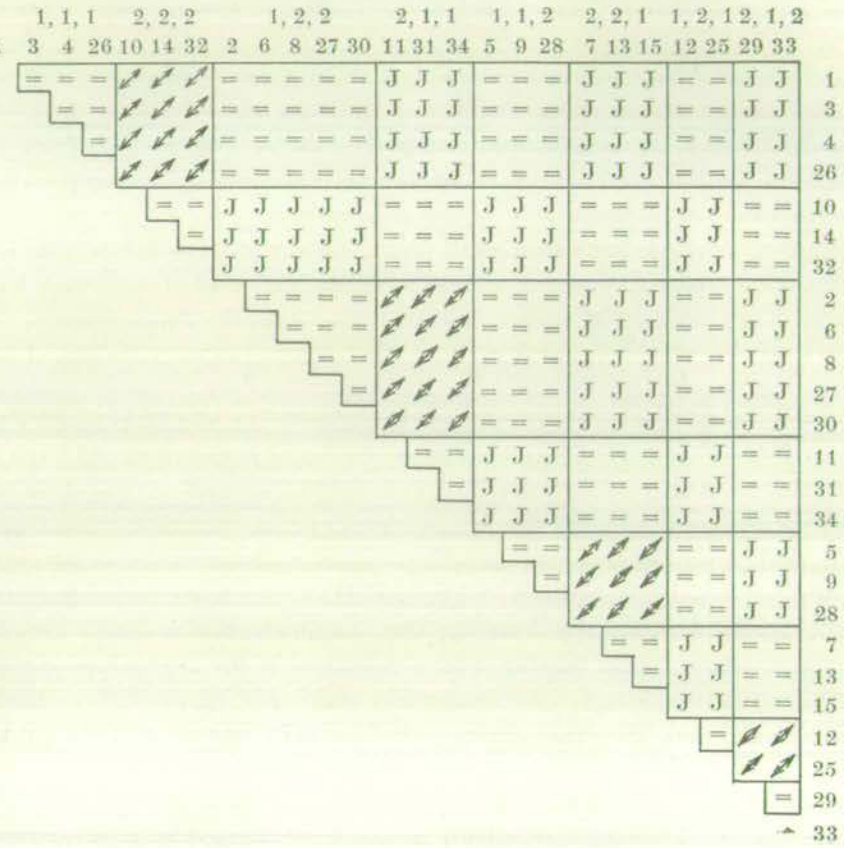
The original isolate was collected by R. F. O. Kemp from horse dung found at Penicuik, Midlothian, in April 1967. The specimen was initially identified as *Psathyrella coprobia* by R. Watling and was placed in the herbarium of the Royal Botanic Garden, Edinburgh, under accession number R.W. 5243. No other isolates belonging to the same breeding group were found. In December 1969 a dikaryotic mycelium was obtained from a mass plating taken from the herbarium. The monokaryolic components of this mycelium were isolated by maceration in May 1970, and shortly afterwards fruit bodies were formed in culture. These were harvested, dried and stored in silica gel. In November 1970 spores from these dried fruit bodies were germinated to initiate the monokaryotic stocks which were used in this study. A total of 73 monokaryons were isolated and these were mated together in various combinations as described below.

4. RESULTS

After mating a random sample of 15 monokaryons in all combinations, seven different mating types were found and not four as expected for a bifactorial species. An eighth mating type was identified when representatives of the seven known mating types were mated with a further sample of monokaryons. A total of 25 monokaryons, including representatives of the eight mating types were then mated again in all combinations and the results are shown in Table 1. The table has been arranged so that strains having the same mating type are next to each other. From the table it can be seen that:

(1) There are four classes of mating in which clamp connexions were isolated

Table 1. *Results of mating 25 monokaryotic mycelia in all combinations*



Dikaryotic mycelia isolated from both sides of the junction line.

J Heterothallic mycelia bearing false clamp connections isolated only from the junction line.

= Matings indistinguishable from the monokaryons.

from both sides of the junction line. This indicated that nuclear migration took place.

(2) There are 12 classes of mating from which heterokaryotic hyphae bearing clamps were isolated only from the junction line. Detailed microscopic observations showed that the clamps were false.

(3) There were 12 classes of mating which were indistinguishable from the monokaryons.

For a single fruit body to produce monokaryons having eight different mating types three factors must be involved in their determination. As the two factors of a bifactorial species have been given the symbols A and B the additional factor present in the trifactorial system has been designated C. Further studies may,

however, reveal that the A and B factors of the two systems are not homologous. Mating types have been assigned to the monokaryons on this basis and the eight combinations of mating type alleles are represented by their numbers only along the top of Table 1. For example 1, 1, 1 represents A1 B1 C1.

In order to make clearer a more detailed analysis of the results shown in Table 1 it is first necessary to consider some of the characteristics of bifactorial incompatibility, which have been revealed by the studies on *Schizophyllum commune* by Raper and his co-workers and of *Coprinus cinereus* (= *C. lagopus sensu* Buller) by Day. In these two species it has been found that heterozygosity for the A factor ($A \neq B =$) results in the formation, at the junction line, of heterokaryotic mycelia bearing false clamp connexions. No nuclear migration takes place and the hyphae which bear false clamps often from a ridge at the junction line which is known as a 'barrage'. When mated strains are heterozygous for the B factor ($A = B \neq$) nuclear migration can occur into both of the established monokaryotic mycelia but the resulting heterokaryotic mycelium has neither true nor false clamps. In *Schizophyllum* these mycelia can be distinguished by their flat morphology (Papazian, 1950).

Table 2. *The genotypes and mating characteristics of bifactorial and trifactorial systems of incompatibility (the symbols used are those of Raper (1966))*

Bifactorial		$A \neq B \neq$ Compatible	$A \neq B =$ False clamps 'Barrage'	$A = B \neq$ Migration 'Flat'	$A = B =$
Trifactorial	$C \mp$	$A \neq B \neq C \neq$ Compatible	$A \neq B = C \neq$ False clamps	$A = B \neq C \neq$ As monokaryon	$A = B = C \neq$ As monokaryon
	$C =$	$A \neq B \neq C =$ False clamps	$A \neq B = C =$ False clamps	$A = B \neq C =$ As monokaryon	$A = B = C =$

On the basis of this evidence Fulton (1950) distinguished between the functions of the A and B factors in a bifactorial system. He concluded that the A factor controls clamp formation and the B factor controls nuclear migration. Although this difference in function seems to be common to the few species which have been studied in detail it is unsafe to generalize too widely as *Coprinus lagopides* has been found to form $A \neq B =$ heterokaryons by migration (Kemp, unpublished).

A comparison between the mating characteristics in bifactorial and trifactorial systems is summarized in Table 2. From the table it can be seen that in a bifactorial system there are four possible combinations of factors and that there are at least three different mating reactions. The functions of the A and B factors can therefore be distinguished. In the trifactorial system there are eight combinations of factors but only three different mating reactions. The two systems are similar in that mycelia bearing false clamps can be isolated from the junction line in the incompatible matings which involve different A factors. However whereas in a bifactorial system only two factors have to be heterozygous for the formation of dikaryotic mycelia throughout both the initial mycelia, all three have to be heterozygous in a trifactorial system. In the bifactorial system heterozygosity for the B factor alone

is sufficient to permit nuclear migration and in *Schizophyllum commune* heterokaryons of the genotype A=B≠ can be distinguished by their flat mycelia. In common A matings of trifactorial systems where either or both of the B and C matings were heterozygous, mycelia with a characteristic growth form were not isolated.

From the above information it can be concluded that in the trifactorial system, as in the bifactorial system, the A factor is largely responsible for the formation of clamp connexions. More information is, however, needed to distinguish further between the functions of the three factors. Further experiments were carried out.

(i) *Nuclear migration experiment*

In order to determine whether nuclear migration takes place in the common A matings of the trifactorial system, eight different common A matings were set up and after the matings had been in contact for several days sample plugs from the peripheries of the colonies were isolated. The sample inocula were each paired with two mating types of genotype opposite to that of the original mating. For example, if the original mating was A1B1C1 × A1B2C2, then the plugs from the peripheries were each mated with A2B2C2 and A2B1C1. Compatible matings resulted only if the peripheral plug was mated with a mating type opposite to that of the original colony. For example, a sample plug from the periphery of the original A1B1C1 colony gave a compatible result only when mated with A2B2C2. This indicated that nuclear migration did not take place between the original mycelia of the common A mating. All three factors must be different for nuclear migration to occur.

During the analysis of mating types it was noticed that sample plugs taken from the various matings had a number of characteristic growth rates. Mycelial growth rate and fruiting experiments were therefore carried out to test this observation in an attempt to obtain more information about the functions of the B and C factors.

(ii) *Mycelial growth rate, and heterokaryon stability*

Two monokaryons were selected and mated in all combinations. Small plugs of mycelia were taken from the junction line of each mating and were inoculated, incubated and measured as described in the Materials and Methods. The colony measurements after 4 days' growth are shown in Table 3. The table shows that the samples derived from the matings which resulted in the formation of clamp connexions were of two sizes. The dikaryons (A≠B≠C≠) and the heterokaryons of genotype A≠B≠C= had a mean diameter of 32·56 and 32·00 mm respectively. The heterokaryons of genotypes A≠B=C≠ and A≠B=C= had a mean diameter of 23·00 and 21·25 mm respectively. The difference between the diameters of the 'fast' and 'slow' clamp bearing mycelia was shown to be very highly significant by an analysis of variance.

Mycelia were isolated from the junction line of all the 120 matings made in the growth-rate experiment and were stored in bijoux bottles. When the mycelia were subcultured after 2 or 3 weeks of storage it was found that in some of the matings

Table 3. *The relative growth rates (mm) of mycelia isolated from the junction-line of a mating between two members of each of the eight mating types*

A1B1C1		A2B2C2		A1B2C2		A2B1C1		A1B1C2		A2B2C1		A1B2C1		A2B1C2		
3	4	10	14	6	8	11	34	5	9	7	13	12	25	29	33	
	26	34	32	26	29	23	26	30	30	30	31	27	28	23	23	3
		30	31	28	30	19	22	31	33	30	30	25	25	21	19	4
			28	19	21	25	26	34	35	34	35	25	24	29	30	10
				19	22	30	30	31	32	30	27	21	25	30	30	14
					25	30	33	26	27	24	20	25	26	33	33	6
						31	34	33	33	26	24	31	32	31	32	8
							28	23	22	31	30	31	32	30	33	11
								25	23	31	29	33	34	29	29	34
									30	33	33	28	28	23	21	5
										34	34	28	28	22	22	9
											30	19	21	27	28	7
												18	23	27	28	13
													30	32	33	12
														31	33	25
															29	29
																33

Fast growing matings in which nuclear formation, true clamp formation and mature fruit body production takes place.

Fast growing matings in which false clamps are formed at the junction line.

Slow growing matings in which false clamps are formed at the junction line.

false clamps were not recovered. False clamps were recovered in all A≠B≠C= matings but not in matings of genotype A≠B=C= and A≠B=C≠. On the basis of these results it is possible to conclude that whilst false clamps are formed when the A factor is different, stable false clamps are formed when in addition the B factor is different.

(iii) *Fruit-body production*

In order to test whether heterozygosity for all three factors is necessary for the formation of fruit-bodies, plates were inoculated with monokaryotic inocula as

described in Materials and Methods. The inocula were arranged according to this pattern because it has been found in certain species of *Psathyrella* and *Coprinus* that fruiting may occur where two monokaryotic inocula come in contact but fails if a plate is inoculated with one or several dikaryotic inocula.

Mature fruit-bodies were formed in five pairs of matings all of which were heterozygous for the three mating type factors. The matings of the type $A \neq B \neq C =$ all developed small primordia but no mature fruit-bodies were formed. All other matings failed to develop any primordia. By referring to Table 3 it can be seen that the fast-growing heterokaryons with false clamps which were isolated from the junction line were the strains which produced primordia, and these all had the genotype $A \neq B \neq C =$. The slow-growing heterokaryons with false clamps which were $A \neq B = C =$ or $A \neq B = C \neq$ developed no primordia. It is therefore possible to conclude that primordium initiation only takes place in matings in which the B factor is different. The heterokaryons with the genotype $A \neq B \neq C =$ although growing at the same rate as the fully compatible matings are distinguishable morphologically by their side branches which often grow in contact with the main axis instead of at an angle of about 45°. This gives the periphery of the colony a rather spiky appearance.

The following conclusions can thus be made. The A factor is concerned with most aspects of clamp formation and is similar in function to the A factor of a bifactorial species. The B factor is concerned with the initiation of primordia while all three factors must be heterozygous for nuclear migration and for the formation of mature fruit-bodies. However, it is still not possible to detect an independent function for C. It is possible that the function of C is epistatic to both of the A and B functions, so that the effect of the C factor would only be detected in compatible matings.

(iv) *The relative position of the three factors on the chromosomes*

In a bifactorial species sister mating occurs at the level of one in four if the factors are not linked and infra-factor recombination is low. All bifactorial species have been found to show independent segregation of the A and B factors. The reduction in the level of sister mating is thought to be the evolutionary advantage of the bifactorial system over a unifactorial one. The halving of sister mating with each additional locus will only occur if the factors segregate independently.

As 73 monokaryotic mycelia were analysed for mating type (Table 4) an indication of the linkage relationships of the three factors is possible. If the three factors are not linked the number of monokaryons in each of the mating type classes should be equal. A chi-square test was therefore carried out to test whether the observed totals in each class differed significantly from the mean of 9·125. The result showed that there was no significant difference from the mean ($\chi^2 = 6{\cdot}67$ with 7 D.F.; $P = 0{\cdot}5 - 0{\cdot}3$). The result for this small sample suggests that the three factors are inherited independently.

Table 4. *The total numbers of monokaryons in the eight mating type classes*

Mating type			Total
1	1	1	8
2	2	2	8
1	2	2	15
2	1	1	8
1	1	2	9
2	2	1	12
1	2	1	6
2	1	2	7
			73

5. DISCUSSION

These results indicate that a system of incompatibility determined by three factors exists in an isolate of *Psathyrella*. Such a system has never before been found in any group of fungi.

Most of the studies of incompatibility in the basidiomycetes have been done on the classical species of *Schizophyllum commune* and *Coprinus lagopus*. It is possible that different systems may be found in other basidiomycetes. Studies in a number of *Psathyrella* and *Coprinus* species show that exceptions from the recognized types of mating behaviour are common. Some species, for example, have no clamp connexions (e.g. *Coprinus congregatus*) although dikaryotic mycelia are formed. But the presence of clamp connexions is not restricted to heterothallic species. Clamps have also been found on the hyphae of several homothallic species. In most species the dikaryotic mycelia are more vigorous than the monokaryons but in a few species of *Coprinus* the monokaryons grow in culture more rapidly than the dikaryons and it is therefore not always possible to isolate compatible dikaryons after migration. The occurrence of nuclear migration in common-B heterokaryons of *C. lagopides* has already been mentioned. In *C. congregatus*, *Psathyrella gracilis* and many other species of *Coprinus* and *Psathyrella* nuclear migration in compatible matings may not occur into monokaryons which have a particular genotype. In this case the restriction in nuclear migration also appears to be controlled genetically but not by the incompatibility factors. In other strains nuclear migration may only be possible over a limited range of temperatures.

Hyphal fusion, nuclear migration and the development of properly formed clamp connexions and fruit bodies may all be controlled by the incompatibility factors. The use of an incomplete scoring procedure has probably accounted for several wrong determinations of incompatibility systems. If no migration information is available and if false clamps are scored as true clamps, then it is possible to misinterpret a bifactorial system as a unifactorial one. Several examples are given below where the incompatibility system of a species has been wrongly determined because false clamps have been scored as normal clamps. Dickson (1936) found *C. macrorhizus* (= *C. cinereus* = *C. lagopus sensu* Buller) to be bifactorial, which agrees with Bensaude (1918), who called the species *C. fimetarius*. However,

Routien (1940) found *C. macrorhizus* to be unifactorial. Similarly Dickson (1934) recorded *C. sphaerosporus* (= *C. lagopides*) as unifactorial but Quintanilha *et al.* (1950) found *C. funariorum* (= *C. lagopides*) to be bifactorial. Recent studies of species in *Coprinus* section *lanatuli*, to which all the above-mentioned species belong, have shown all the described species and several new ones to be bifactorial. Misidentification is therefore unlikely to account for these differences in mating behaviour. Some more examples where incompatibility systems of a species have been wrongly determined are cited by Aschan (1954).

The results given in this paper also show that it is important to test for all aspects of incompatibility and not just for the appearance of clamps. It also seems likely that the function of mating type factors in the basidiomycetes as a whole may not necessarily be similar to those found in the classical bifactorial species.

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TWO NEW COPROPHILOUS SPECIES OF PSATHYRELLA

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TWO NEW CORPROPHILOUS SPECIES OF PSATHYRELLA

ROY WATLING & MARIA JURAND*

ABSTRACT. Two new species, *Psathyrella fimetaria* Watling and *P. coprophila* Watling are described from Scotland. Cultural characteristics are given and their biological significance discussed. A key to the coprophilous species of *Psathyrella* found in the British Isles is given.

INTRODUCTION

With few exceptions, species of *Psathyrella* are generally regarded as difficult to identify because of lack of literature and disagreements as to the interpretation of the often inadequate old descriptions. It was never thought, however, that the coprophilous members of this genus were to be included in this category for they are few in number and fairly well-documented. One of us (R.W.) during a survey of the Isle of Rhum, North Ebudes, found an unknown species of *Psathyrella* growing on pony dung. It was widespread on the island and extremely common in all areas frequented by these animals. Later the same species was found on horse-dung in several localities in the immediate area of the Kindrogan Field Centre, Perthshire.

Since these collections were made it has been found in other localities in Scotland. A collection from Midlothian made by R. F. O. Kemp, which was microscopically similar, was assumed to be referable to the same species. However, cultural characters of the two species were found to be at variance, and a detailed micro-anatomical analysis was made resulting in the finding of several differences between the Midlothian collection, and the Perthshire and Rhum specimens. The Midlothian material has been successfully induced to produce fruit-bodies over several generations. The purpose of this paper is to formally describe the two species as new and discuss various cultural characteristics. It would be safe to say that on classical criteria alone, few would have suspected that two distinct species were involved but now that the differences have been demonstrated there would appear to be little difficulty in separating the two.

TAXONOMY

(R. Watling)

1. Psathyrella fimetaria Watling, **sp. nov.** Fig. 1.

Pileus 5–12 mm, e conico vel conico-convexo conico-expansus vel expansus late umbonatus, castaneus vel badius, sicco pallide-ochraceus vel argillaceo-albidus, ad discum obscuriore coloratus ochraceo-tinctus, jove pluvio striatus, jove sicco leviter ruguloso-atomatus primo, ad partem externam squamulis fibrillosus ad marginem residuis veli fugacibus dispensis. *Lamellae* late adnatae e pallido- vel purpureo-umbrinae, subconfertae ad aciem albidae vel interdum albido-flocculosae. *Stipes* 40–55 × 2–3 mm, aequalis

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vel ad basim leviter interdum flexuosus, albidus vel sursum pallide sordido-brunneolus, ad apicem albo-pruinosus, ad basin albo-vel albido strigulosо-fibrillosus. *Caro* tenuissimo, pilei concolorata, stipites albido. *Sporae* ellipsoideae, poro germinative, 13·5–14·5(–15) × 6·5–7·5(–8) μm. *Basidia* 4-sporigera. *Cystidia* aciei lamellarum praecipue 25–30 × 10·5–15 μm, ad apicem 5·5–7 μm. *Cuticula pilei* cellularis.

Typus: Scotland, Isle of Rhum, *Watling* 7355

Pileus 5–12 mm (up to 14 mm high), conical to convex then expanded, very rich chestnut-brown or bay-brown at first, darker at disc, hardly darkening with age although becoming flushed sepia, striate at margin at first then ½ to ¾ way with age, rapidly drying to become atomate and pale greyish buff to ochraceous; margin with silvery-white veil fibrils. *Stipe* 40–55 × 2–3 mm, equal or slightly swollen at base, whitish throughout only slightly darker towards base at maturity, slightly subtomentose at very base where attached to substrate. *Gills* adnate, sepia-pallid at first, then flushed purplish sepia, soon purple-black, with slightly paler or whitish flocculose margin, slightly mottled as spores mature. *Flesh* white in stipe slightly ochraceous or watery honey in pileus, paler when dry. *Veil* copious at margin of pileus when very young and on stipe, soon becoming lost or adpressed when on stipe.

Spore-mass purple-chestnut. *Basidiospores* ellipsoid in face-view, slightly flattened in side-view, 13·5–14·5(–15) × 6·5–7(–8) μm, thick-walled, dark vinaceous brown with hint of umber in water, unchanged by ammoniacal solutions or Melzer's reagent, although decolorised by concentrated sulphuric acid; apiculus small but distinct, germ-pore obvious c. 1 μm broad, central. *Basidia* 4-spored, 22·5–25 × 11·0–12 μm, clavate, monomorphic, hyaline in water, hardly coloured in KOH or ammoniacal solutions. *Cheilocystidia* numerous, hyaline in water and ammoniacal solutions, elongate lageniform or narrowly lageniform. *Pleurocystidia* present, hyaline, similar to narrowest cheilocystidia, or fusiform, 37–40 × 10·5–11·5 μm (apex—4·5–5 μm), hyaline in ammoniacal solutions. *Pileal surface* composed of broad irregularly rounded, ellipsoid to obovate cells with distinctly brownish coloured walls; *pileocystidia* absent. *Caulocystidia* variable up to 45 μm long × 5·5–10·5 μm, in small clusters, elongate lageniform—subcapitate to clavate, hyaline in water and ammoniacal solutions. *Pileus-trama* of irregularly rounded, ellipsoid to ovate cells, 11·5–18·5 μm broad with strongly coloured walls, flattened and elongate towards the darker base. *Hymenophoral trama* homogeneous with regularly arranged swollen cells, brownish in both water and alkali solutions, dark red-brown in ammoniacal solutions in central zone and immediately beneath pileus-trama. *Clamp-connections* infrequent, only seen in cells of stipe. *Stipe cortex* of elongate cylindric cells, c. 10 μm broad with brownish or dark ochraceous coloured walls. *Veil* very sparse and of elements 3·5–5·5 μm broad.

On horse and pony dung, solitary or in small groups, sometimes attached in twos and threes at base, Isle of Rhum, 25 viii 1964, *Watling* 7355 (Type) & *Watling* 7356; Kilmory, Isle of Rhum, 26 viii 1964, *Watling* 7357; 17 viii 1967 & 3 ix 1968, Kindrogan, Perthshire, *Watling* 7440; 22 viii 1970, Straloch, Perthshire, *Watling* 7441 & 7470.

Recognised by lack of pink-edged gills, presence of pleurocystidia, a copious veil when very young and size and shape of basidiospores.

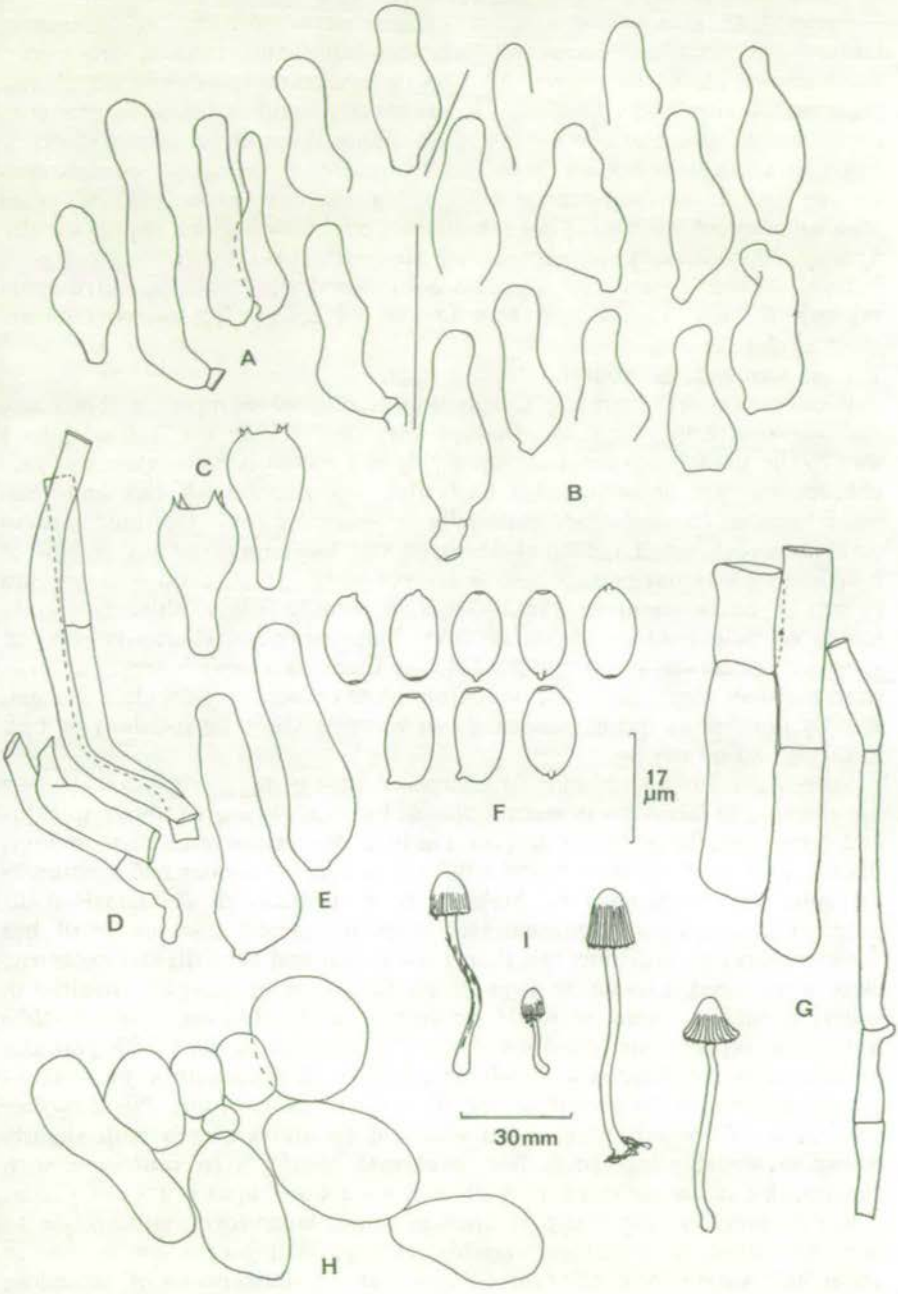

FIG. 1. *Psathyrella fimetaria*: A, caulocystidia; B, cheilocystidia; C, basidia; D, veil-elements; E, pleurocystidia; F, basidiospores; G, cortical cells of stipe; H, pileal surface; I, habit-sketch. Magnification of all microscope structures as indicated.

2. Psathyrella coprophila Watling, **sp. nov.** Fig. 2 & Plate 3.

Pileus 5–18 mm (18 mm altus) campanulatus, conicus vel convexus, badius, ochraceo-ferrugineus vel ochraceo-bubalinus, striatus prostremo, sicco leviter atomatus primo, ad partem externum squamulis fibrillosus, residuis veli fugacibus. *Lamellae* late adnatae ex pallidae dein fuscae, subconfertae, ad aciem albido vel interdum albido-flocculosae. *Stipes* 50–60 × 2–3·5 mm, aequalis vel ad basin leviter interdum flexuosus, albidus dein sursum pallide sordido-brunneolus, ad apicem albo-pruinosus, ad basin albo vel albido fibrillosus. *Caro* tenuissimo, pilei concolorata, stipitis albido. *Sporae* ellipsoideae, poro germinativo distincte, 12–13(–14) × 5·5–6·5 × 6–7 μm. *Basidia* 4-sporigera, *Cystidia* aciei lamellarum praecipue utriformia vel lageniformia, 18·5–3·5 × 10·5–12 μm, ad apicem 4·5–6 μm. *Cuticula pilei* cellularis.

Typus: Scotland, Edinburgh, *Watling* 3947.

Pileus 5–18 mm (18 mm high), campanulate, conical to convex, commencing bay, chestnut honey or tawny flushed horn-colour with age, striate ¼ to ½ way to the slightly darker disc, rapidly drying out to become atomate, pale ochraceous rust or ochraceous buff with a slight greyish tint and then resembling a *Panaeolus* sp. especially in monokaryotic fruiting; margin with numerous small indistinct fibrils of veil extending as faint groups of hyphae to ½ way but remnants soon disappearing. *Stipe* 50–60 × 2–3·5 mm (5 mm at base), equal or slightly swollen, whitish silver, shining, slightly honey or pale reddish brown at silky fibrillose base where attached to substrate, pruinose at apex. *Gills* fuscous black to purplish sepia, at first, soon purplish black, adnate with slightly paler or whitish flocculose margin, slightly mottled as spores mature. *Flesh* white in stipe, horn-colour or buff in pileus and on drying.

Spore-mass fuscous black. *Basidiospores* 12–13(–14) × 5·5–6·5 × 6–7 μm ellipsoid in face-view in general outline but slightly angled about apiculus and germ-pore, flattened in side-view and hilar depression often very obvious, thick-walled, dark purplish black with hint of umber in water and ammoniacal solutions, unchanged in Melzer's reagent although decolourised to purplish amethyst with concentrated sulphuric acid; apiculus small but obvious, germ-pore distinct less than 1 μm broad and very slightly excentric. *Basidia* 4-spored, clavate 28·5–30 × 10–12 μm, monomorphic, hyaline in water, hardly coloured in KOH or ammoniacal solutions. *Cheilocystidia* numerous, utriform or broadly and shortly lageniform, filled with granular material and ornamented with oily droplets above venter, 16·5–35 × 10·5–12 μm. *Pleurocystidia* absent or very rare up to 25 μm long. *Pileal surface* composed of irregularly rounded ellipsoid to obovate cells with slightly brownish walls; *pileocystidia* few, obclavate, utriform to ventricose with short neck and obtuse head, 18·5–21 × 8·5–10·5 μm, apex c. 4·5 μm. *Caulocystidia* numerous especially at apex of stipe, lageniform, subcapitate to narrowly utriform or clavate, variable, 13·5–47 × 4·5–13·0 μm, hyaline in water and ammoniacal solutions. *Pileus trama* homogeneous of vesiculose cells up to 20 μm broad with or without slightly brownish walls in water and alkali. *Hymenophoral trama* homogeneous, composed of regularly arranged swollen cells, hyaline in water or slightly brownish in ammoniacal solutions. *Clamp-connections* present, numerous on cortical cells of stipe. *Veil* remnants on pileus of hyaline, filamentous cells, 3·5–8 μm broad with

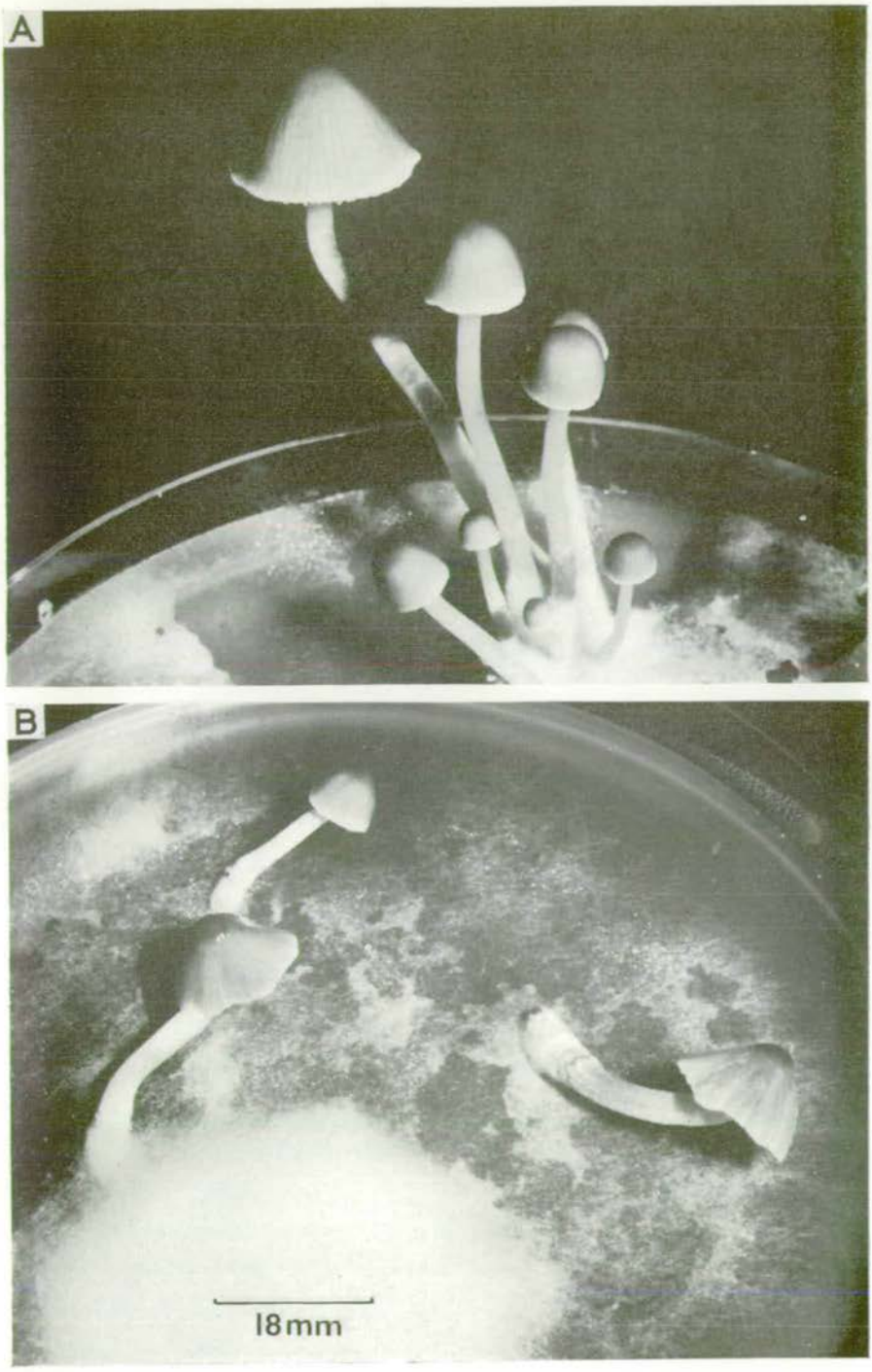

PLATE 3. Fruit-bodies of *Psathyrella coprophila* grown in culture: A, dikaryotic fruit-bodies; B, monokaryotic fruit-bodies. Size as indicated.

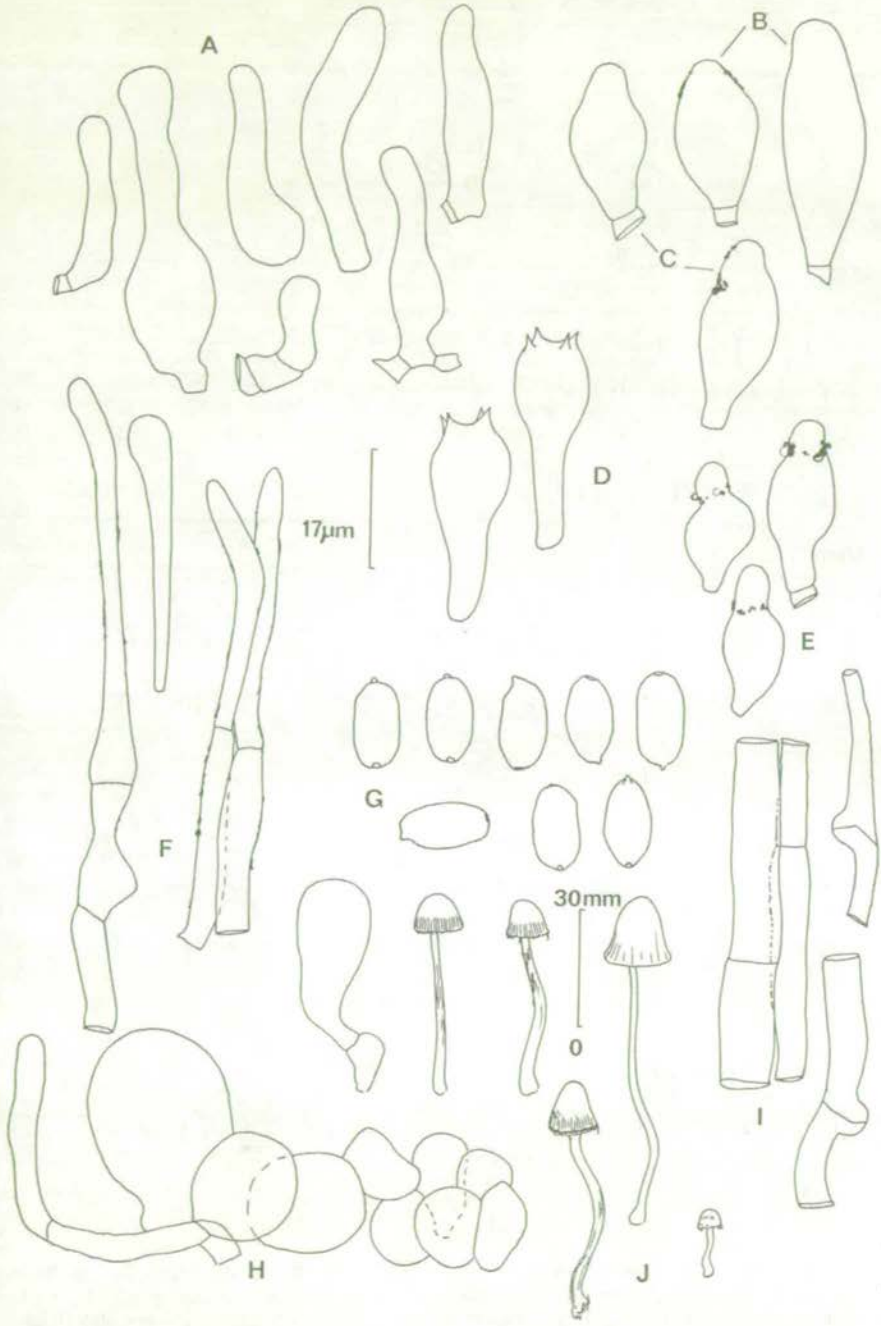

FIG. 2. *Psathyrella coprophila*: A, caulocystidia; B, cheilocystidia; (from culture) C, cheilocystidia (from original collection); D, basidia; E, pleurocystidia; F, veil-elements; G, basidiospores; H, pileal surface; I, cortical cells of stipe; J, habit-sketch. Magnification of all microscope structures as indicated.

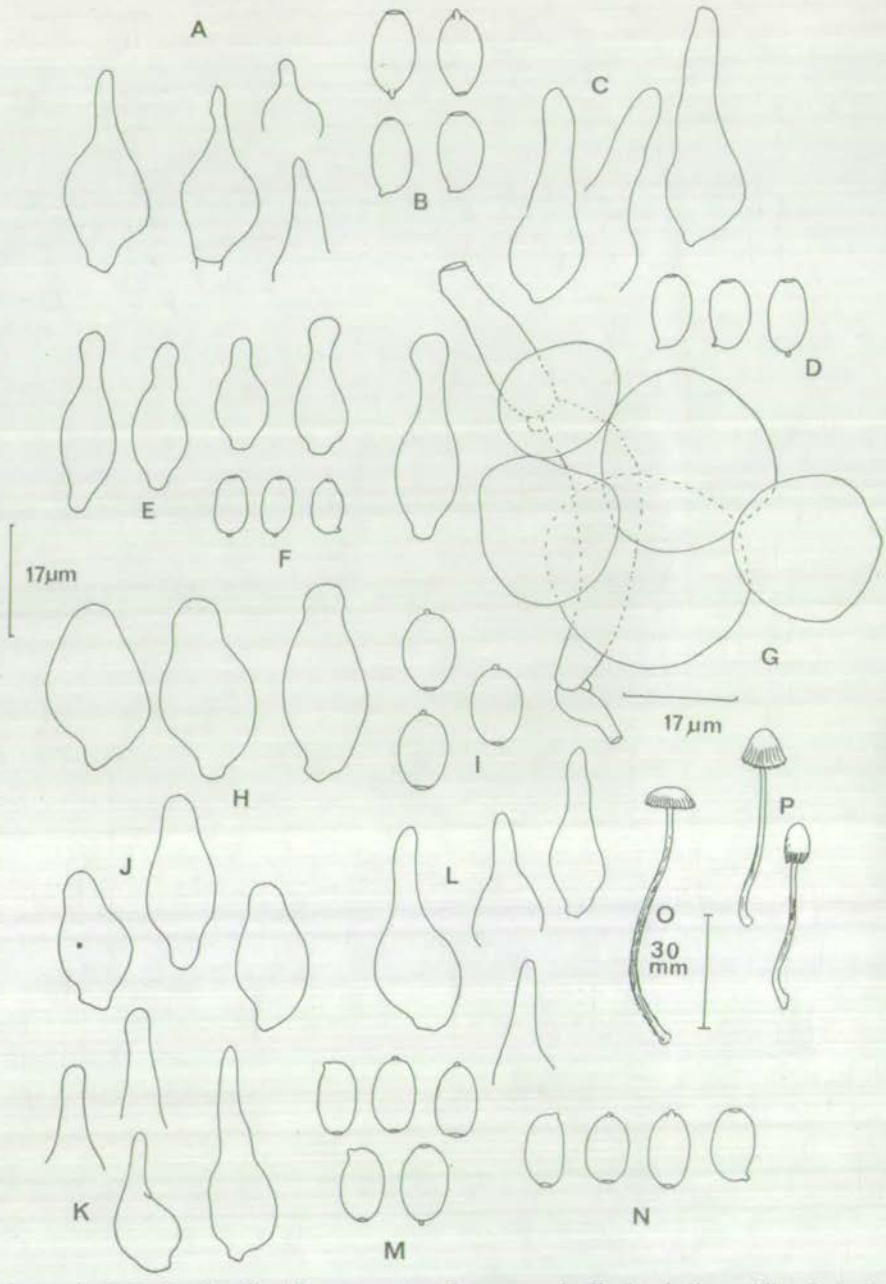

FIG. 3. A & B, *Psathyrella* aff. *stercoraria*: *Orton* 2193 in E; A, cheilocystidia; B, basidiospores; C, D & O, *Psathyrella stercoraria*, *Watling* 7359; C, cheilocystidia; D, basidiospores; O, habit-sketch; E–G, *Psathyrella sphaerocystis*: (type—*Orton* 2193); E, cheilocystidia; F, basidiospores; G, veil-constituents; H–J & P, *Psathyrella* aff. *coprophila*, *Watling* 5069; H, cheilocystidia; I, basidiospores; J, pleurocystidia; P, habit-sketch; K–N, *Psathyrella coprobia*; K, cheilocystidia & N, basidiospores (from *Watling* 7358 Scotland); L, cheilocystidia & M, basidiospores (from *Watling* 3514, Idaho U.S.A.). Magnification as indicated.

slightly irregular wall ornamentation. *Stipe cortex* of elongate, cylindric cells 4–8 μm broad.

On horse dung, solitary or in small groups, Blackford Glen, Edinburgh, 1 xi 1966, *Watling* 3947, legit *R. F. O. Kemp* (subsequently in culture, *Watling* 5063, 5064 and 6020).

P. coprophila freely produces fruit-bodies in Petri-dishes on agar-surfaces but these are more variable than those found in nature. The largest sized fruit-bodies resemble the size of *P. fimetaria* collected on Rhum. *P. fimetaria* is normally more of a robust species.

P. coprophila can be distinguished by the white margin to the gills, thin, fugacious white veil which is very easily lost on handling or at maturity, size of basidiospores and honey to tawny pileus-colour. It is separable morphologically from *P. fimetaria* in size, basidiospore-shape and sparsity of pleurocystidia.

Discussion

The spore-size and the dark colour of the spore-print place both these species in *Psathyrella* subgenus *Psathyrella*. Both these newly proposed species lack the pink-edged gills typical of *P. stercoraria* Kühn. & Joss. Both species differ from *P. coprobia* by the lack of the copious, white flocculose veil and paler pileus-colour, although they would appear to be fairly closely related. The Rhum collections were at first referred to Smith's interpretation of *Psathyrella coprobia* (J. Lange) A. H. Smith as outlined in the notes accompanying the '*New Check list of British Agarics and Boleti*' (Dennis et al., 1960). However, referring to Smith's original description and his personal notes, the emphasis placed on basidiospore-size by the British authors would appear too great. One of us (R.W.) has collected typical *P. coprobia* in the western United States in the presence of A. H. Smith, and the European (Fig. 3 K & L) and American (Fig. 3 M & N) concepts agree.*

Searching the literature has failed to find a name for either of our fungi. The recently described *P. sphaerocystis* P. D. Orton (Fig. 3 E–G) differs markedly in the presence of a mealy-granular veil. *P. stercoraria* (Fig. 3 C, D & O), which has been seen recently in Scotland, is also unrelated in the characters of the cheilocystidia, although it is similar in the size of the basidiospores and the fugacious veil remnants; it is a very small fungus. The presence or absence of a pink-edged gill is a difficult character to determine and almost impossible in dried material. However, fruit-bodies at all stages of development have been available for both *P. fimetaria* and *P. coprophila* and no evidence of a red-line at the gill-edge, at any stage during the life-history, has been found.

Key to the British species of Coprophilous Psathyrella

1 Veil composed of globose, ellipsoid or vesiculose cells; basidiospores 8–9 × 4·5–5·5 μm . . *P. sphaerocystis* P. D. Orton, 1964

\+ Veil when present composed of filamentous cells; basidiospores over 9 μm long 2

* *Watling* 3514, Hughes Fork, Upper Priest River, Idaho, U.S.A. (E).

2 Basidiospores over 6 μm wide (13–16 × 7–8 μm); pileus ochraceous brown soon becoming alutaceous or ochraceous cream, or ivory; gills brownish . *P. albidula* (Romagn.) Moser, 1955

\+ Basidiospores rarely wider than 6 μm, or if up to 6·5 μm then 13 μm or less in length 3

3 Pileus convex to semiglobate, red-brown and entirely covered in white, floccose veil remnants; basidiospores 10–12 × 5·5–6 μm . . . *P. coprobia* (J. Lange) A. H. Smith, 1941

\+ Pileus campanulate, smooth but for occasional remnants of veil when young 4

4 Gill-margin coloured reddish; pileus small up to 8 mm broad *P. stercoraria* Kühn. & Joss.,* 1957

\+ Gill-margin not red, white or only slightly paler than gill-face . 5

5 Basidiospores 10·5–11·5 × 8–8·5 μm *P.* aff. *coprophila* Watling (Fig. 3 H–J)

\+ Basidiospores 12 μm or over in length, less than 8 μm in breadth 6

6 Pleurocystidia present; basidiospores 12–13(–14) × 5·5–6·5 × 6–7 μm . . . . . *P. coprophila* Watling

\+ Pleurocystidia scarce; basidiospores 13·5–14·5(–15) × 6·5–7·5 (–8) μm . . . . . *P. fimetaria* Watling

Culture studies

(M. K. Jurand)

In both *P. coprophila* and *P. fimetaria* all mycelia were grown from the spores of one fruit-body only. The fruit-body of *P. coprophila* was the type specimen from Midlothian and the fruit-body of *P. fimetaria* was collected in Perthshire (*Watling* 7470).

All descriptions and experimental studies were made on mycelia grown on agar plates at 20°C on horse dung extract medium (Lange 1952).

1. P. coprophila Watling

Macroscopic characters. Monocaryotic mycelial colony growing to 37–46 mm diameter in 7 days, mainly submerged, almost transparent, later producing varying amounts of aerial growth, then appearing sparsely woolly, often patchy; aerial hyphae frequently with glistening droplets. Margin of colony regular.

Dikaryotic mycelial colony growing to 60–65 mm diameter in 7 days, with strong radial growth lines; aerial hyphae less frequent than in monokaryons. Margin of colony regular.

Microscopic characters. Basidiospores germinating within 24 hours to form an irregularly branched colony.

Monokaryotic mycelium with no clamp-connections; hyphal diameter at first branch 2·9–3·7 μm. Angle of side branches 45°–65°. Oidia cylindrical 3·0–4·5 × 1·2 μm, borne on aerial hyphae in wet heads, which later coalesce to form droplets.

* A collection made by Orton related to this species is illustrated in Fig. 3 A & B (Surlingham, Norfolk, 27 vii 1960, *Orton* 2193). This collection, however, differs in size of fruit-body and several anatomical details.

Dikaryotic mycelium with clamp-connections at most septa; hyphal diameter at clamp-connections 3·7–5·0 *μm*. Angle of side branches 25°–45°. Dikaryotic hyphae only rarely producing monokaryotic side branches which bear oidial heads.

Fruiting in culture. Dikaryons form primordia after about 14 days of incubation in the light and mature fruit-bodies after about 20 days. (Plate 3A).

Some monokaryotic strains form monokaryotic fruit bodies after three to four weeks of incubation in the light (Plate 3B).

A comparison of dikaryotic and monokaryotic fruit bodies in *P. coprophila* is summarised below.

	Dikaryotic	*Monokaryotic*
Colour of cap	snuff-brown	fulvous
Size of cap	5–15 mm	5–10 mm
Length of stipe	20–50 mm	10–35 mm
Posture of stipe	erect	almost prostrate
Colour of gill	cigar brown	fawn
Colour of gill edge	cigar brown	fawn
Spore density	abundant	few
Colour of spore print	fuscous black	—
Spore size	12·5–14·0 × 6·5 *μm*	12·5–14·0 × 6·5 *μm*
Spore no. per basidium	4	4
Pleurocystidia	absent or infrequent	abundant
Cheilocystidia	present	present

Most of the differences shown above are the direct effect of differences in spore-density. The main diagnostic character of a monokaryotic fruit-body is the absence or low density of spore-print. The only difference which is independent of spore density is stipe posture. The stipes of dikaryotic fruit-bodies are firm and erect while those of monokaryotic fruit-bodies lack directed growth. This is thought to be due to a difference in their response to gravity.

2. P. fimetaria Watling

Macroscopic characters. Monokaryotic mycelial colony growing to 16–25 mm diameter in 7 days, mainly submerged almost transparent; aerial hyphae few. Margin of colony regular.

Dikaryotic mycelial colony growing to 35–38 mm diameter in 7 days. Growth mainly submerged at first, then woolly aerial hyphae produced abundantly in patches. Margin of colony regular.

Microscopic characters. Basidiospores germinating to produce at first a long unbranched hypha, later a sparsely branched colony.

Monokaryotic mycelium with no clamp-connections, hyphal diameter at first branch 3·3–4·2 *μm*. Side branches fewer than in *P. coprophila*, set at an angle of 50°–70°. Oidia cylindrical, borne on submerged hyphae; aerial droplets of coalesced oidial heads formed less frequently than in *P. coprophila*.

Dikaryotic mycelium with clamp-connections at most septa; hyphal diameter at clamp-connections (2·9)–3·3–4·2 *μm*. Angle of side branches 50°–70°.

Fruiting in culture. No mature fruit bodies were obtained, but primordia occasionally formed in cultures contaminated with bacteria.

Experimental studies

Breeding Systems. Both *P. coprophila* and *P. fimetaria* are heterothallic and have mating alleles at a single locus (bipolar). *P. coprophila* was shown to be a bipolar species by Kemp (personal communication) by crossing strains grown from several basidiospore tetrads. The spores of each tetrad were isolated with a micromanipulator. The breeding system of *P. fimetaria* was shown to be bipolar by using monokaryotic mycelia grown from basidiospores obtained from a spore-print. Ten monokaryotic mycelia were cross-mated in all combinations.

Nuclear Migration. In many Basidiomycetes a monokaryotic mycelium can be rapidly dikaryotised by the migration of nuclei into it from a dikaryotic inoculum. Dikaryotisation also occurs when two compatible monokaryons come into contact.

An experiment was designed to test whether migration of nuclei from a dikaryon into a monokaryon occurs in *P. coprophila* and *P. fimetaria*. In each species six different monokaryons, each replicated twice, were used with the same dikaryon. The monokaryotic colonies were allowed to grow till the plates were almost covered with mycelium. The original inoculum was then removed and replaced by a dikaryotic inoculum of the same species. To test the rate of nuclear migration, plugs 5 mm apart were taken every 24 hours, and were examined later for the presence of clamp-connections. In this way the distance travelled by the dikaryotizing nuclei was estimated.

It was found that the nuclei of *P. coprophila* migrated, on average, the radial distance of 47 mm in 7 days, a rate approximately 1·5 times as great as the growth rate of the dikaryon. The nuclei of *P. fimetaria* did not migrate at all even by the fifth day of sampling.

If these results are indicative of what happens in other populations of both species in nature then their implications may be considerable. In natural populations of *P. coprophila* nuclear migration might allow an established monokaryon to be dikaryotized by a secondarily established mycelium of the same species, thus giving rise to a mycelium mosaic. In natural populations of *P. fimetaria* the mycelia probably remain more individually distinct. This difference in migration potential may have played a part in the evolution of the two species.

Intersterility between the species. The intersterility of *P. coprophila* and *P. fimetaria* was tested by mycelial mating and oidial homing.

a) *Mycelial mating.* Four monokaryons, two of each mating type, of each species were crossed in all combinations. No dikaryons were formed.

This indicates that representative populations of two species are intersterile when allowed to meet in the artificial environment of the agar plate. In nature this genetical isolation would have permitted morphological divergence of the two taxa.

b) *Oidial homing.* Oidial homing, first observed by Kemp (1970) is a reaction between hyphae and oidia of the same species. When oidia are smeared on the edge of a monokaryotic colony the hyphal tips grow towards the oidia and later fuse with them. The directed growth of the hyphal tips

towards the oidia can be clearly seen under the low power of the microscope and the whole test can take less than one hour.

An experiment was designed to test the hyphal homing in both species using strains of different mating types. The results showed that reciprocal tests between the species never gave any reaction but that within both *P. coprophila* and *P. fimetaria*, monokaryotic hyphae fuse with the oidia of their own species regardless of mating type. Dikaryotic hyphae can also home, but the reaction is less distinct than that of a monokaryon.

The results of the homing experiment agree with those of the mycelial mating experiment. Both suggest that *P. coprophila* and *P. fimetaria* are reproductively isolated.

Conclusion

The results show that experimental studies can be of use to the fungal taxonomist, both as a source of characters and as a means of confirming the validity of taxa. In this case the type of breeding system and the presence of nuclear migration may be used as taxonomic characters along with morphological ones. The mycelial mating and homing experiments have been used to test the biological status of the two taxa. Their unambiguous results independently confirmed the conclusions reached by morphological considerations.

Acknowledgments

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